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ABSTRACTS OF PAPERS PRESENTED AT THE TWENTY-NINTH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, RICHMOND, VIRGINIA, DECEMBER 27 TO 30, 1938, INCLUSIVE

Studies on Septoria bromigena. J. L. ALLISON. Of 34 species of *Bromus* inoculated in the field and greenhouse with *Septoria bromigena*, only *B. inermis* was susceptible, and inbred lines of this species differed greatly in susceptibility at the Waseca Branch Station (Minnesota) in 1938. On susceptible lines, *S. bromigena* causes a destructive leaf spot, and occasionally, also attacks the stems and panicles. The range of temperature for infection is between 15° and 25° C. The germ tubes penetrate the epidermis directly. The organism grows slowly in culture, producing sparse mycelium and abundant pycnidia. There are decided differences in cultural characters among the many monosporous lines isolated. Mycelial sector variants have been observed in all lines, those tested have remained distinct, and, for the most part, have been nonpathogenic.

Effect of Nutrient Variations on Host and Parasite in the Rhizoctonia Stem Rot Disease of Bean. E. J. ANDERSON. In the course of studies of characters associated with resistance in plants to attack by root- and stem-rotting organisms, pursued as a National Research Council Fellowship activity, the effect of nutrient variations on the severity of attack on bean roots and stems when inoculated with *Rhizoctonia solani*, as well as on the organism itself were observed. Bean plants grown in sand culture with a continuous flow of solutions of high nutrient salt concentration were markedly more severely attacked than were those grown in solutions of low nutrient concentration. In general, solutions that were favorable to host growth and severe attack were also favorable, upon addition of dextrose, to the growth of the fungus in liquid culture. However, the nutrition of the fungus previous to inoculation appeared to have no effect on the severity of attack on the host, provided approximately equal amounts of the inoculum were used.

The Factorial Interpretation of Anthracnose Resistance in Beans. C. F. ANDRUS. A system of 10 Mendelian factors in 3 allelomorphic series, involving both duplicate and complementary dominant genes for resistance, 1 dominant gene for susceptibility and interactions between genes in different, as well as the same, allelomorphic series, is necessary to explain all the data obtained separately with 2 physiologic races of bean anthracnose (*Colletotrichum lindemuthianum*) on intercrosses of 10 parent varieties. The same system of genes suffices to explain the data for both forms of the pathogen, although the assumed genotype of any one variety is not the same in respect to both forms. That all 10 varieties should be of different genotype is beyond the range of probability where only 36 genotypes are possible according to the hypothesis.

Pathogenicity Experiments with Isolates of Fusarium vasinfectum Causing Cotton Wilt. G. M. ARMSTRONG, J. D. MACLACHLAN, and R. WEINDLING. Ten monosporial isolates from different localities were used in 1937 with the cotton varieties Farm Relief, Semi-Wilt, and Super-Seven. One recent isolate and 4 cultural variants were added in 1938, and all were used with Farm Relief and Dixie Triumph 12. Two-gallon pots containing non-sterilized soil were heavily infested with cultures of the pathogen on oat-wheat mixture. The isolates ranged from very virulent to practically non-pathogenic. In general, their degree of virulence was of a similar order in the 2 years towards the susceptible Farm Relief. Two of the cultural variants had lost much of the pathogenicity of the isolates from which they originated; the other 2 had not. Reisolation substantiated, in general, the wide range of virulence expressed by external symptoms. Most isolates producing abundant aërial growth on nutrient agar were in the virulent group; otherwise, no definite connection between virulence and cultural characteristics was established. The degree of resistance of many cotton varieties is not constant in all localities. This phenomenon may be explained in part by the foregoing evidence of differences in pathogenicity between isolates of the fungus.

Movement of the Virus of Tobacco Mosaic. C. W. BENNETT. In vegetative Turkish tobacco plants, with a main stem in horizontal and a basal sucker in vertical position, basipetal movement of virus was rapid; acropetal, slow. In plants, otherwise similar but with maturing seeds, the reverse was true. In vegetative stems, acropetal movement was accelerated by darkness or defoliation. Basipetal movement was very slow in main stems in dark. In *Nicotiana glauca* plants with top and basal Turkish tobacco grafts separated by 3 feet of stem, virus moved from the top to basal graft and produced symptoms in 6 to 9 days. In 7 of 10 plants, virus failed to move from basal to top grafts in more than 200 days. When tops were defoliated, upward movement was relatively rapid. Tobacco-mosaic virus moved through rings in Turkish tobacco that broke phloem continuity, but passage was delayed. In certain *N. glauca* plants, virus passage in similarly-ringed stems did not occur in periods greater than 250 days. Cucumber-mosaic virus passed such rings in all cases. This evidence indicates that movement of tobacco-mosaic virus is correlated with food transport. Factors involved are probably not fundamentally different from those responsible for movement of other plant viruses.

The Effect of Insect Juices on the Infectivity of Plant Viruses. L. M. BLACK. Juice of the clover leaf hopper, *Aceratagallia sanguinolenta*, carrying potato yellow-dwarf virus, failed to produce any lesions when rubbed upon *Nicotiana rustica* L. Juice from yellow-dwarf *N. rustica* plants was rendered noninfectious by adding leaf-hopper juice. Juices of other insect vectors interfere with the infectivity of other plant viruses. Since yellow-dwarf virus is unstable, the inhibitor in leaf-hopper juice was studied through its action in reducing infectivity of tobacco-mosaic virus. Reduction in infectivity follows immediately upon addition of the inhibitor to the virus and is independent of the time inhibitor and virus are in contact. Percentage reduction in infectivity is dependent chiefly upon the concentration of the inhibitor, and is affected very little by concentration of the virus. Juice from 0.15 mg. of clover leaf hoppers, containing about 0.0015 mg. of protein nitrogen, reduces the infectivity of 1 cc. of a tobacco-mosaic-virus solution by 50 per cent. The inhibitor is nondialyzable, thermolabile, and unstable in acid or alkaline solution. The infectivity of certain mixtures of tobacco-mosaic virus and inhibitor can be increased by dilution or by heat treatment. Infectivity of tobacco-mosaic virus in practically noninfectious mixtures was completely restored when the inhibitor was removed by ultrafiltration or ultracentrifugation.

Decay of Hardwoods by Ustulina Vulgaris and Other Ascomycetes. DOROTHY J. BLAISDELL. The black crustose stromata of *Ustulina vulgaris* have been observed the world over on stumps, logs, and tree wounds. Recently it has been repeatedly isolated from butt rot of hardwoods by members of the Division of Forest Pathology. The presence of it and occasionally other ascomycetes in decayed wood prompted testing them in pure culture for their ability to cause decay. They were grown on autoclaved and hot-water-dipped blocks of green sugar maple, beech, and black oak sapwood in Kolle flasks at room temperature. The extent of the decay caused by the various fungi was judged from the respective percentage losses in weight of the oven-dried wood. In 6 months *U. vulgaris* isolated from rotted sugar maple caused a loss in weight of 18 per cent in beech, 11 per cent in sugar maple, and 26 per cent in black oak. Under less favorable moisture conditions an ascospore culture from black oak caused 6 per cent, 8 per cent, and 17 per cent loss, respectively. The typical white decay with black zone lines was produced. *Xylaria polymorpha, X. fusca, Daldinia concentrica, Nummularia atropunctata, Hypoxylon* sp. and *Strumella coryneoidea* produced considerable decay. *Xylaria, Daldinia*, and *Nummularia* produced about as much as *Ustulina*. Inoculations with *Ustulina* in living oak produced typical decay in heartwood in one year. Results of inoculations in many other hardwood species are not yet available.

The Response of Phymatotrichum omnivorum to Heavy Metals and Other Elements. LESTER M. BLANK. The effect of Cu, Fe, Mn, Zn, Al, Bo, Co, Cd, F, Hg, I, Li, Mo, Ni, and Si on the root-rot organism, *Phymatotrichum omnivorum*, has been studied in a nutrient solution purified or nonpurified by the calcium carbonate treatment. The factorial design and analysis of variance were used. They have permitted many comparisons and interpretations that otherwise would have been obscured. In purified solution the addition of Fe, Mn, or Zn at various concentrations gave marked increases in mat weight. Copper was beneficial at low concentrations but was toxic at 10 or more parts per million, except in certain special combinations with Fe and Zn. In nonpurified solutions the addition of any copper to the iron-manganese-zinc combination resulted in depression of growth. Fe, Mn, and Zn were beneficial, particularly so, in certain combinations. The addition of the other elements (Al, Bo, etc.) at moderate concentrations to nonpurified or purified solutions containing optimum amounts of iron, manganese, and zinc failed to give significant increases in mat weight. Nickel and cobalt were toxic, even at low concentrations.

Natural Water-soaking and Bacterial Infection. ARMIN C. BRAUN and JAMES JOHNSON. Periods of high soil and atmospheric moisture in Wisconsin in 1938 permitted field observation of water-soaked foliage resulting from internal pressure. This condition was seen on many plant species. In tobacco seed-beds especially a close relation was found between the amount of such water-soaking and the extent of natural infection and invasion with *Bacterium angulatum*. Water-soaking can be induced experimentally by placing heavily watered potted plants at 100 per cent relative humidity. Under such conditions 42 out of 51 plant species were water-soaked. Often only small areas water-soaked, but frequently as much as 30 per cent of the leaf area was affected. Tomatoes sometimes showed water-soaking in ten minutes but most species required 2 hours or more. The rate and extent of water-soaking is influenced by the salt concentration of the soil, apparently because of its effect on the osmotic value. Potassium nitrate added to the soil in proper concentrations not deleterious to plant growth inhibited water-soaking to a striking degree as compared to control plants, suggesting a possible preventive measure against disease. Experimentally, *Bacterium angulatum* invaded a water-soaked leaf for a distance of 3 inches from a single point of infection in a period of 30 hours.

Two Distinct Viruses from the Mosaic Complex in Lilium longiflorum. PHILIP BRIERLEY. From typical flecked mosaic Easter lilies, a virus of the cucumber mosaic group may be isolated in cucumber, from which it may be inoculated into tobacco, lily, etc., and recovered by the usual rubbing methods. Inoculations from flecked lilies into tulips produce mottling and flower breaks in the following season. No symptoms are produced on inoculating cucumber from such broken tulips, but inoculation from these tulips to lily seedlings produces mottling in 10 days. Inoculations from strongly mottled Easter lilies without fleck symptoms produce breaking in tulips, but no symptoms in cucumber. Inoculations from symptomless Easter lilies (except seedlings) also produce breaking in tulips, but no symptoms in cucumber. Host-range inoculations have thus far failed to reveal any common host other than lilies for the cucumber and tulip components of lily mosaic. *Lilium formosanum* seedlings, inoculated by the usual rubbing technique, develop distinct mottling in response to the tulip component, or components, in 10 days; mottling is less distinct in response to the cucumber component. The cucumber-mosaic strain (similar to Price's) and the strong mottle virus (related to McWhorter's latent virus of lily) differ in properties also.

Marsh Spot of Peas Caused by Manganese Deficiency. HELENA L. G. DE BRUYN. Two series of water and sand cultures of different pH and containing different quantities of manganese sulphate proved that manganese deficiency really causes the necrosis of peas, known as marsh spot. Carefully washed quartz sand, distilled water, and salts of analytic grade were used. Sand cultures of pH about 8.2 did not produce a single ripe pod without manganese; they produced 90 per cent diseased peas with 2.5 or 5 mg. manganese sulphate per pot, but only 2 per cent with 15 mg.; those of pH about 6.3 developed pods even without any manganese sulphate. Of these and of those grown with 0.05 or 0.1 mg. of manganese sulphate per pot, 98 per cent had marsh spot; with 2 mg. per pot, only 5.5 per cent had it. The more alkaline water cultures without or with, 0.025, 0.05, or 0.1 mg. manganese sulphate per liter produced no seeds, or 100 per cent diseased peas; with 1 mg., 20 per cent. The more acid solutions without, or with, 0.025 or 0.05 mg. also produced no seeds or 100 per cent marsh spot; with 0.5 mg., all peas were healthy. Naturally, more manganese sulphate is needed in the more alkaline solutions than in the more acid ones to produce healthy peas.

Daedalea unicolor on Maples and Other Hardwoods. W. A. CAMPBELL. *Daedalea unicolor*, usually considered a saprophyte, causes butt and trunk rots, generally associated with cankers of sugar and red maples in New England. It occurs less frequently on yellow and white birches and roadside elms. It is most prevalent on trees of sprout origin and infects vigorous dominant trees as often as suppressed. Infection is most common through dead stubs or dead stems of multiple-stem clumps. Roadside trees are infected through wounds and sunscald areas. The rot is yellowish in the early stages and white, soft in the advanced stage. The fungus rots the stem center faster than the sapwood margin, but eventually it grows outward through the sapwood apparently killing the cambium and causing a pronounced canker, with each yearly extension marked by faint to prominent callusing. It may fruit extensively on the cankered surface, but trees usually break over from the weakening effect of the rot before it fruits. In that case it fruits profusely on the down trunk and dead snag. At times *D. unicolor* may kill infected trees by girdling. In a sprout clump it spreads readily from stem to stem eventually killing all. If sprouts are connected only at ground level, it spreads through the living sapwood at the base of the stems.

Sterile Conks of Polyporus glomeratus and Associated Cankers. W. A. CAMPBELL and ROSS W. DAVIDSON. Sterile conks or knots of fungus tissue were studied on a number of beech trees at several localities in New England during the past summer. They had formed on cankered areas where no branch stub or injury occurred, at branch stubs surrounded by cankered areas, or, in still other cases, at branch stubs with no associated cankers. Normal fruiting bodies of the fungus were never found on living trees, but many cultures obtained from the sterile conks and from associated decay were uniformly similar and, in macroscopic and microscopic characteristics, were the same as stock cultures previously obtained from normal sporophores of *Polyporus glomeratus*. No attempt was made to determine the percentage of beech trees infected with this fungus, but trees bearing cankers and sterile conks were found, on dissection, to be extensively decayed. In several stands of red maple a high proportion of the trees, most of which were cankered, were infected with *P. glomeratus*. Cankers are often an indication of infection of red maple by this fungus, but sterile conks are absent or so inconspicuous as to be unnoticed. It is apparent that normal fruiting bodies develop only after infected trees die or are broken over by wind. They form on the outside of the bark of the dead snags or more commonly on the fallen trunks.

Effect of Temperature on Infection and Development of Eight Physiologic Races of Puccinia graminis tritici on Wheat Seedlings. ROBERT C. CASSELL. Races 11, 21, 34, 36, 38, 49, 56, and 59 of *Puccinia graminis tritici* produced no uredia on wheat seedlings inoculated, incubated, and kept at 2° C., although flecks appeared within 21 to 30 days. Only races 38, 49, 56, and 59 survived for 30 days at 2° C. Race 38 alone survived in the host for 85 days at 2° C. and later continued to produce pustules on the host in the greenhouse. At a slightly higher temperature, race 56 thrived much better than race 38. In comparative tests between races 36 and 56 on Ceres wheat, the greatest difference seemed to be in the amount of infection at different temperatures. Race 36 caused the heaviest infection at moderate to low temperatures, while race 56 caused the heaviest infection at moderate to high temperatures. Race 15 survived 14 days at an average temperature of 39.6° C. and a maximum of 55.0° C. Mycelial survival has been observed in the host for 85 days at low temperatures, and for 42 days at high temperatures.

(Cooperative investigations, U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station.)

Effect of Temperature on Urediospore Germination and Germ Tube Development of Five Physiologic Races of Puccinia graminis tritici. ROBERT C. CASSELL. A study was made of the effect of temperature (2°, 9°, 20°, and 30° C.) on germination of urediospores and development of germ tubes of races 11, 34, 36, 38, and 56 of *Puccinia graminis tritici*. In general, 20° was most favorable, followed in order by 9°, 30°, and 2°. However, the races did not all behave alike. Spores of race 34 germinated better than those of the other races over a wide range of temperature, but germ tube growth was poorest at 20° C., the general optimum. Germ tubes of race 56 developed faster than those of all others at 20° C., closely followed in this respect by races 11 and 38. In the basis of urediospore germination, race 36 was one of those best adapted to high temperatures and was only partially tolerant to cold; race 56 was next to 36 in its ability to develop at high temperatures and was the least adapted to cold; and races 38 and 11 were best able to tolerate low temperatures.

(Cooperative investigations, U. S. Department of Agriculture and Minnesota Agricultural Experiment Station.)

Source of Leaf-rust Inoculum for Fall Infection of Wheat. K. STARR CHESTER. Wheat leaf rust in Oklahoma might conceivably oversummer: (1) in volunteer wheat or grasses, (2) as urediospores in the soil, or (3) in the alternate host, *Thalictrum* spp. All seem unlikely as major sources of fall inoculum, because: (1) very little leaf rust was found on grains and grasses from June to November 1938, (2) the urediospores do not appear to be adapted to withstand the extreme fluctuations of temperature and moisture to which the soil is exposed during these months, and (3) leaf rust has never been collected on *Thalictrum* in Oklahoma. Results from airplane spore traps indicate that the principal source of fall inoculum is air-borne urediospores. North winds in late October were heavily laden with leaf rust urediospores (150,000 spores per 1,000 acre feet). The samples also showed an abundance of smut spores, but contained relatively few types of spores that might have been produced locally at that time of year; and this, in the light of our knowledge of air drift, implies that the spore storms have not originated in the Southwest. It appears the most likely explanation that the fall wheat is infected mainly by urediospores produced in the most northern wheat-growing regions.

Heat Treatments of Black Locust for Root-knot Control. K. STARR CHESTER and MAX CRESS. A general root knot infestation in a large locust nursery raised the question of salvage through heat disinfestation. 200 well-infested seedling trees were heated in water at various temperatures for various intervals. Each was then planted in sterilized soil, and okra, tomato, and cowpeas, were planted in the soil surrounding each tree. Knots were first noted in control indicator plants after 25 days. Final readings (38th day) showed successful disinfestation without injury to the trees in 30 minutes at 118°, 120°, and 122° F., in 12 minutes at 120° and 122°, and in 5 minutes at 122°. Beginnings of injury were observed in trees heated to 124° (5, 12, or 30 minutes). At or below 116° (30′), 118°

(12′), and 120° (5′) the indicator plants showed infestation. Treating at 120° for 30′ will probably prove the most serviceable procedure with black locust, since it allows a fairly wide margin of safety. Trees so treated did not reinfest themselves, but did continue to produce root nodules in sterilized soil. The heated trees were somewhat more vigorous than unheated checks. Trees heated at 130° to 160° produced small vegetative shoots that quickly died, evidently because water and foodstuffs contained in the stems were exhausted.

Fungicidal Studies with Special Reference to the Vegetable Oils. E. E. CLAYTON and H. H. FOSTER. In studies conducted with the blue mold of tobacco, *Peronospora tabacina*, it has been found that oils differ widely in their fungicidal value. Emulsified and applied as sprays, Eucalyptus, pine, paraffine, chaulmoogra, castor, palm, cocoanut, olive, and beef-tallow oils were ineffective or very slightly effective. Cotton, corn, rapeseed, linseed, peanut, sesame, tung, cod-liver, and sperm oils had marked fungicidal value. The fungicidal value of the better oil emulsions was increased by the addition of copper as Bordeaux mixture, basic copper sulphate, copper resinate, copper oxychloride, and cuprous oxide. Best results were obtained with cuprous oxide. The fungicidal value of the oil-copper combination was further increased under some conditions by the addition of benzol, xylol, and paradichlorbenzene.

White Root Rot of Apple Trees (Corticium galactinum). J. S. COOLEY and ROSS W. DAVIDSON. A rather virulent apple-root rot seems to have escaped notice since 1902, when Von Schrenk referred it to *Thelephora galactina* Fr. (now a synonym for *Corticum galactinum*). Affected trees rapidly succumb, but evidence so far indicates that the disease is not highly contagious. Trees inoculated with a single-spore culture as they were transplanted showed 100 per cent infection, but inoculated trees, undisturbed in the nursery, have shown no more than 50 per cent infection. The surface of affected roots is covered with a dense weft of fungus mycelium. The bark of the root and finally the cambium and wood are penetrated and killed in irregular patches. When the bark is removed, these areas appear as lighter spots with darker borders. This symptom may be still further intensified if the host develops callus tissue around the killed areas, giving a knotty and warty effect. Eventually, the entire root is killed. The fruiting stage of the fungus (hymenium) is white in the early stages and later turns to a buff or ochraceous color. It is formed in cavities in the soil around infested roots or occasionally about the root collar of affected trees. Plants of dewberry, blackberry, Japanese wineberry, dogwood, *Lychnis alba*, and sumac have been found infected when growing near apple trees having the disease.

The Hawkesbury Watermelon, a Promising Wilt-resistant Variety. HAROLD T. COOK and T. J. NUGENT. Further trials with the Hawkesbury watermelon in 1938 demonstrated that it is a suitable wilt-resistant variety for commercial use. In heavily contaminated soil in the greenhouse four strains of the Hawkesbury variety averaged 17.3 per cent seedling wilt as compared with 52.2 per cent for the Leesburg, 81.8 per cent for the Owens Grey and 74.3 per cent for the Tom Watson variety. Under commercial conditions the Hawkesbury produced melons that averaged 35 pounds in weight and some of which weighed as much as 45 pounds. Total sugars averaged 8.7 per cent and in some melons ran as high as 9.7 per cent. The melons grown in the experimental plot were smaller, because of less favorable growing conditions, but corresponded to those grown under commercial conditions in other characteristics. They were oblong in shape and light greenish gray in color with fine dark green veins. The rind was ¾ to 1 inch thick and extremely tough. The flesh was pink to deep pink or red in color, tender, sweet, and free of white heart. A survey showed that a majority of the growers who tried the Hawkesbury this year found it suitable for their use.

Two Years Experiments in the Control of Cherry Leaf Spot (Coccomyces hiemalis). ROBERT H. DAINES. Experiments were conducted in New Jersey during the growing seasons of 1937 and 1938 in which the following fungicides were compared for the control of cherry leaf spot (*Coccomyces hiemalis*): lime sulphur, lime sulphur plus aluminum sulphate and lime; wettable sulphur; wettable sulphur plus orthex; 1–3–50 and 2–3–50 Bordeaux mixtures, made with high calcium and high magnesium lime; 1–3–50 Bordeaux plus metallic zinc; home-made and commercial copper phosphates, with and without bentonite; coposil, with and without orthex; Z–O; Cupro-K; oxo-bordeaux; basicop, copper hydro 40; orchard spray 34 plus zinc sulphate; cuprocide 54; and aluminum sulphate and lime. Most of the commercial coppers mentioned above were used with and without lime. In these tests the copper fungicides gave more satisfactory control of leaf spot than did the sulphurs. Aluminum sulphate and lime plus lime sulphur was somewhat better than lime sulphur alone, and wettable sulphur plus orthex was much better than wettable sulphur alone. During wet periods, some of the copper fungicides gave considerable injury, especially where lime was omitted. Lack of injury in some cases was explainable by poor adhesive properties of the fungicide. Fungicidial efficiency and adhesive properties of the coppers were generally correlatable. Bordeaux mixture in any combination produced considerable injury to cherries.

A Study of the Yellow Mosaics of Potato. T. P. DYKSTRA.[1] Pseudo-net-necrosis virus secured from Holland, and tuber blotch virus obtained from Ireland were found to be identical. Tubers from infected plants showed internal patch-like necrosis. Aucuba mosaic produced only a variegated type of mottling in American potato varieties, but caused considerable foliar necrosis in the English variety, British Queen. An unknown disease received from Canada and tentatively designated as Canada-Streak, produced severe foliar necrosis in addition to a yellow mottling on the lower and middle leaves of every variety tested. Serological and protective studies proved it to be a necrotic strain of Aucuba mosaic. The thermal inactivation point, longevity in vitro, and the reaction to different pH values of the above named viruses were very similar. Calico produced irregular to cream-color spots on the leaves of infected potato and pepper plants. Tubers from diseased calico plants did not show any internal symptoms. Infected potato and pepper plants failed to protect against infection of any of the other above mentioned viruses. On the basis of the results secured, it appears that pseudo net-necrosis, Aucuba mosaic and Canada-Streak are closely related, whereas there is no relationship between this group and Calico.

Tripsacum dactyloides, Another Native Host of Aplanobacter stewarti. CHARLOTTE ELLIOTT and ALICE L. ROBERT. (Submitted by title.)

Psorosis in Relation to Other Virus-like Effects on Citrus. H. S. FAWCETT. Recent studies have revealed that a number of trunk, branch, and fruit effects in citrus, formerly described as different diseases, are associated with symptoms on young growing leaves that so far have not been distinguished from each other nor from the young leaf symptoms associated with true psorosis. Experiments have shown that the young leaf symptoms of each of these effects may be transmitted by budding, as in case of psorosis. This would indicate the occurrence of a group of strains of one virus or a variety of closely related viruses, all of which may have a similar effect on the young leaf, but each having a different effect on some other tissues of the plant. The different effects presenting this same symptom in young leaves, in California, are psorosis A, psorosis B, concave gum disease, blind pocket, corky bark, and crinkly leaf. Since certain symptoms of psorosis A and B on mature leaves and fruit are similar to zonate chlorosis and cyclosis in Brazil, and some types of leprosis in Florida, Argentine, and Brazil, and concentric ring blotch in South Africa, these also are suspected to be related virus diseases.

Early Planting, an Aid in the Control of Onion Smut. E. L. FELIX. Partial and sometimes marked control of onion smut is obtained in the Elba, New York, muck section by sowing the crop very early (usually, March 20 to April 5), as soon as the season permits. Onion seed germinates at a slightly lower soil temperature than smut infection occurs. The minimum temperature for onion seed germination and growth is about 8° C., and that for smut growth and infection, about 10° C. Prevailing soil temperatures of 8° to 13° C. during preemergence appreciably decrease smut. Maximum temperatures, ranging mostly from 8° to 10° C. in the top inch of soil, reduce smut in the greenhouse and field from 60 to 78 per cent of that occurring at the optimum temperatures of 15° to 20° C. Very early planting is suggested as a supplement to the regular method of control with formaldehyde, particularly in fields with a comparatively small amount of smut. Early planting of onions is conducive to large yields, aside from reduction of smut, although somewhat greater risk from wind injury is incurred. Late plantings, when the soil temperature exceeds the optimum for infection, decreases smut more erratically because of frequent drops in temperature, especially at night.

Yield Reduction by Lime Sulphur on Apple Trees. DONALD FOLSOM. In a young McIntosh apple orchard in Maine, 338 trees were studied for 11 years in 45 plots replicated in 5 series. The 5 series have received respectively: (1) dry lime-sulphur spray every year; (2) lead arsenate 8 years and dry lime sulphur spray 3 years; (3) minimum of lead arsenate for leaf-chewing insects; (4) fine-sulphur spray; and (5) sulphur dust. The corresponding average trunk girths attained are 35.5, 35.7, 35.2, 36.7, and 35.0 cm., which gives a range of only 5 per cent with the fine-sulphur spray and sulphur dust at the extremes. The corresponding yield rates per tree in 1938 were 11, 13, 34, 43, and 47 lb., and, in 1933 to 1938, inclusive, were 47, 65, 78, 104, and 123 lb. The 50 per cent reduction, in yield rate to date, by lime-sulphur spraying as compared with sulphur spraying and dusting, is highly significant, according to statistical analyses of plot and tree data.

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[1] Associate Pathologist, Division of Fruit and Vegetable Crops and Diseases, B. P. I.

Immunization of Sugarcane as a Basis for Determining Validity of Virus Classification. I. L. FORBES. On the basis of symptoms produced, several strains of sugarcane-mosaic virus have come to be recognized in Louisiana. In a series of inoculation experiments during 1937 and 1938 it has been found that one mosaic virus may give rise to different symptoms on different hosts, that different cane varieties do not have equal susceptibility to the same virus and that the presence of one virus within a host may immunize the host against infection from subsequent inoculation. Moreover, some mosaic viruses fail to immunize the host against subsequent infection by certain other viruses. It follows that immunity studies afford a convenient means of checking the validity of virus classifications based upon symptoms alone.

Production of Setae by Colletotrichum falcatum in Culture. I. L. FORBES. So far as is known, the red-rot fungus, *Colletotrichum falcatum*, has not been observed previously to produce setae in artificial culture. During the course of preliminary experiments designed to test the antagonism of *Trichoderma* and *Actinomyces* isolates to *C. falcatum* it was noted that setae were produced abundantly by the anthracnose fungus in the region in which *Trichoderma* came in contact with *C. falcatum*. These observations were made on 14-day bean-agar cultures.

Effect of Environment on Metabolism of Tomato Plant as Related to Development of Blossom-end Rot of the Fruit. A. C. FOSTER. Extensive growth and chemical composition data were collected from 8 crops of tomatoes grown under controlled greenhouse conditions involving differences in soil moisture, soil nutrition, air temperature, light duration, and the interrelation and interaction of these environmental components, any one of which may become a limiting factor to normal plant growth. The data suggest that the factors affecting the development of blossom-end rot are far more complex than is usually recognized. High-carbohydrate plants had the largest percentage of the disease when grown under short-day conditions; high-nitrogen plants were very susceptible to the disease under long-day conditions; soil moisture at 70 per cent of saturation was optimum for vegetative growth and also for the disease; superphosphate had a marked effect in reducing the incidence of the disease; the rate of transpiration or water requirement apparently had no relation to the disease as it appeared at both extremes of high and low transpiration when other conditions were favorable for its appearance; any one component of the environmental complex may become a limiting factor to the normal activity of all others and promote conditions favorable to the disease.

Physico-Chemical Studies on the Tobacco-Mosaic Virus Protein. VERNON L. FRAMPTON. A new viscometer has been devised that may be used in viscosity determinations under pressure gradients as low as 15 dynes per cc. This viscometer has been used in physico-chemical studies on the tobacco-mosaic virus protein. It has been observed that at higher rates of shear the protein sols approximately follow Poiseuille's law, but at lower rates of shear, the sols show a rather striking anomaly. This anomaly has been observed at all concentrations of virus protein used, and at several pH values of the buffer solutions. Viscosity studies also have been made of the virus protein dispersed in urea and glycine solutions. The anomalous behavior of the protein in dilute aqueous solutions of electrolytes and the results from diffusion studies indicate that the chemically prepared virus protein is not molecularly dispersed in these aqueous solutions, but that the protein particles are in reality aggregates.

The Fungicidal Activity of Phenothiazine and Some of its Oxidation Derivatives. M. C. GOLDSWORTHY and E. L. GREEN. Several years of experimental work at the U. S. Horticultural Station at Beltsville, Maryland, have demonstrated that phenothiazine, used at the rate of 2 lb. to 50 gal. of water, with or without adjuvants, is of value as an orchard spray against apple scab. Laboratory studies show that it is also toxic against the peach brown rot and apple bitter-rot organisms. Laboratory studies of the oxidation derivatives, phenothiazine-sulphoxide, phenothiazone, and thionol, indicate that the active principle of phenothiazine is phenothiazone, and that phenothiazone is toxic in dilutions as low as 2.5 p.p.m. by volume of water. Limited phytocidal studies show that the material may be safely applied to pear, apple, plum, cherry, grape, rose, lilac, and bean foliage. Peach foliage is slightly injured by the treatments.

Fusarium Species Associated with Diseases of Cereals in Manitoba. W. L. GORDON. A taxonomic study of the *Fusarium* species found associated with diseases of cereals in Manitoba was commenced in 1932. Approximately 85 per cent of the collections of diseased basal parts of wheat, oats, barley, and rye, made during 1932–35, yielded *Fusarium* species. The 12 species and 9 varieties of *Fusarium* isolated represented 7 Sections of the genus. Representatives of the Sections Roseum, Gibbosum, Elegans and Discolor were most frequently isolated. *F. avenaceum*, *F. equiseti*, *F. oxysporum* v. *aurantiacum* and

F. culmorum accounted for approximately 90 per cent of the total number of isolations. Leaves of durum wheat were found in one instance heavily attacked by *F. poae.* Infrequent isolations from head blight of cereals yielded 6 species of *Fusarium* belonging to the Sections Sporotrichiella, Discolor, Gibbosum and Roseum. *F. poae* occurred most frequently. In a general study of fungal invasion of seed samples of wheat and barley, *Fusarium* species were isolated from approximately 58 per cent of the samples of common wheat and barley and 47 per cent of durum wheat. The 10 species of *Fusarium* isolated belonged to the Sections Gibbosum, Sporotrichiella, Discolor, Liseola and Elegans. *F. scirpi* v. *acuminatum, F. equiseti, F. poae* and *F. culmorum* were the most commonly isolated species.

Systemic Brooming of Robinia Pseudoacacia and Other Virus-like Diseases of Trees. THEODORE J. GRANT. Systemic brooming of black locust has been in the United States for at least 70 years and is distributed from southern Pennsylvania south to northeastern Georgia and west to southwestern Ohio and Tennessee. The disease has been transmitted only by grafting and budding. Symptom expressions range from extreme brooms to mild brooms, to vein-cleared leaflets with tapered bases. Infected but healthy-appearing plants sometimes develop symptoms when cut back or defoliated. Brooms result from abnormal development of normally located buds into short succulent branches bearing greatly reduced leaves and leaflets. Development of these axillary buds into branches is accompanied by partial defoliation. In dormant stems stored starch is inversely roughly proportional to the severity of brooming. Root pieces from visibly diseased trees are less vigorous than those from healthy ones. As compared with those of healthy plants, the root systems of potted diseased plants were shorter and darker, and had excessive rebranching of rootlets that gave the appearance of root brooms. Systemic brooming diseases with somewhat similar aboveground symptoms have been found on honey locust, white ash, and black walnut. Other virus-like symptoms have been found on European black ash, papaw, oaks, and *Sophora.*

The Epidemiology of Seed borne Microorganisms in Cereals. F. J. GREANEY and J. E. MACHACEK. A study of grain samples collected from many localities in Manitoba showed that kernel infection by fungi is extremely common. This infection was found to arise from air-borne spores lodged on the kernels, the percentage of infected kernels depending on the stage of host maturity at the time spore showers occurred. The samples differed greatly in the amount of infection; those from adjacent fields showing as much difference as those from widely separated localities. Particular attention was given to the development of a satisfactory technique for the estimation of kernel infection. Centrifuging seed washings and analysing the deposit gave a good estimate of surface contamination, but many tests of different disinfectants and culture media were necessary before a combination good enough to give a satisfactory estimate of internal infection was discovered. It was found that many different species of fungi and bacteria infect the seed, while yeasts often predominated in the surface flora. To establish the value of each sample for seed purposes the percentage of germination and the amount of seedling blight were determined in autoclaved soil in the greenhouse, as well as under ordinary field conditions.

Observations on the Supposed Colloidal State of Sulphur in Fused Bentonite Sulphur. A. B. GROVES. Microscopic examination of dispersing and suspended particles of fused bentonite sulphur in water indicates that the major part of the sulphur is not in the colloidal state, but actually present in rather large particles. These observations are substantiated by sedimentation and extraction technique. All material actually colloidal, whether sulphur or bentonite, is quickly precipitated by lime as commonly used in preparing the spray suspension, leaving no colloidal material in the spray after it is ready to be applied.

Particle Size of Elementary Sulphur Fungicides. A. B. GROVES. The size of individual sulphur particles of several spray and dusting sulphurs has been closely estimated and recorded through the combined use of photomicrographs taken at carefully determined magnifications and double printing with an artificial screen of much greater accuracy than that of the best available wire sieves. The 325 Standard Series sieve is much too coarse for use in determining particle size in sulphur fungicides, because it gives no indication of actual particle size with its 44-micron openings. The limitations of the microscope in determining the significant size and surface exposure of the individual particles of a sulphur fungicide are such that measurements made with the microscope are but little more accurate than those made with the simple method reported above. Photomicrographs of proprietary sulphurs show a wide variation in the size of the sulphur particles in these products.

A Red Forcing Tomato Resistant to Cladosporium Leaf Mold. E. F. GUBA. A red tomato for commercial greenhouse culture of satisfactory yield and quality and resistant to *Cladosporium fulvum* has been developed at the Waltham, Massachusetts sub-station from original hybrids of *Lycopersicon pimpinellifolium* the resistant parent, and *L. esculentum* varieties Success, Belmont, and Break O'Day, and from subsequent crosses of progeny of these hybrids, pure lined for resistance, with Waltham Forcing. The new tomato, a special selection of the latest hybrids, has been named Bay State.

The Effect of Various Soil Amendments on the Development of Club Root (Plasmodiophora brassicae) of Crucifers. C. M. HAENSELER. Soils infested with *Plasmodiophora brassicae* were treated with equivalent weights of several calcium, magnesium, potassium, and sodium compounds in dosages equivalent to 1000 and 2000 lb. CaO per acre. Soil pH readings were taken at monthly intervals and cabbage grown as test crop. Nontreated plots showed 100 per cent very severe clubbing. Perfect control of infection was not obtained in any case, but some reduction in infection and marked decrease in plant injury were shown on all plots where the soil pH was maintained at 7.0 or above, regardless of whether the corrective used contained potassium, sodium, calcium, or magnesium. Carbonates, except sodium, gave better results than oxides or hydroxides. Greatest reduction in infection, but serious plant stunting, occurred on sodium carbonate plots. These, with pH readings ranging from 7.4 to 8.2, gave 54 to 74 per cent clean and 20 to 35 per cent severely clubbed plants. A potassium carbonate plot with pH ranging from 7.2 to 8.5 showed 32 per cent clean and 57 per cent severely clubbed plants. Carbonates of calcium and magnesium produced slightly lower pH readings than carbonates of sodium and potassium and gave only 4 to 7 per cent clean and 59 to 62 per cent severely clubbed plants, but showed marked improvement in plant growth.

Cultural Studies on a Species of Entomophthora from the Apple Leaf Hopper (Typhlocyba pomaria). J. G. HARRAR, L. I. MILLER and S. A. WINGARD. Single conidiospores of an *Entomophthora* species, isolated from diseased apple leaf hoppers, and cultured in egg-yolk medium, produced filaments, secondary conidia, hyphal bodies, and resting spores (zygospores). The growth characteristics of the colonies varied with the media on which they were grown. This organism is not readily distinguished from the other species of the genus *Entomophthora* by means of morphological characters; but when compared, on the basis of physiological characters such as fermentation of sugars and protein hydrolysis, the forms tested were found to react differently. These results suggest physiologic criteria that might be of value in differentiating the members of the genus *Entomophthora.*

Physiologic Races of the Fungus Causing Bean Rust. L. L. HARTER. In 1935 the occurrence of 2 physiologic races of bean rust (*Uromyces phaseoli typica*) was established. Since then a study has been made of bean-rust material collected or received from various parts of the United States and Hawaii. From the collections examined, 13 distinct physiologic races have been isolated. No morphological differences between the races can be recognized, the differentiation being based wholly on reaction to differential hosts. The bean-rust survey has disclosed that two or more physiologic races may occur in a single locality the same year, usually with one race predominating in ratios as high as 10 to 1. A race that predominated for any given year may not necessarily do so the following year, but may become subordinated to another race of a much larger population. There is no stability of rust races with respect to geographical locations, the different races shifting geographically from year to year.

Dr. W. J. Zaumeyer and Mr. C. F. Andrus have assisted in these investigations.

The Clonal Variety for Tree Planting: Asset or Liability? CARL HARTLEY. Forest pathologists have long viewed with alarm the planting of pure stands of single species. A stand composed of a clonal variety is the pure stand to the nth degree. In forest and shade trees a clonal planting is even purer than in orchards, since the former are ordinarily on their own roots. The expectation that genetic uniformity will favor the building up of specialized strains of parasites is supported by practical experience with such clonal cultures as Lombardy poplar avenues, rubber plantations, fruit trees, roses, potatoes, bananas, sugar cane, and the creeping-bent golf-green grasses. A promising attempt to grow Norway poplar in Ohio for pulp has been frustrated by a specialized bark fungus. Other recent examples are cited. To utilize hybrid vigor or other desirable qualities in species in which they are most easily obtained by vegetative propagation, and yet have plantings that can adapt themselves both to disease attack and to varied local conditions, it is proposed that mixtures of desirable clones be employed rather than blocks or avenues of a single clone.

Yellow Cuprous Oxide as a Fungicide of Small Particle Size. J. W. HEUBERGER and J. G. HORSFALL. Three types of cuprous oxide powders with a color gradation from purple through red to yellow as the particle size diminished were laboratory-tested for fungicidal value and greenhouse- and field-tested for seed- and foliage-protective value. The order of magnitude for fungicidal value for the 3 types was 1, 2, and 8, respectively, and the dosage by weight for maximum seed-protective value on peas in the greenhouse and climate laboratory was 1, ½, and ¼ per cent, respectively. At ¼ per cent dosage on peas in the field, emergence was 74 and 82.4 per cent, respectively, for the red and yellow types. The order of magnitude of foliage-protective value against rose black spot in the field was 1 and 3 for the red and yellow types. With equivalent copper concentrations on tomatoes the number of leaves per plant killed by *Alternaria*, 35 days after the last application, was 129, 84, 63, and 39 for the nonsprayed and the 3 types, respectively. Likewise, the percentage infected fruit was 9.9, 7.7, 6.6, and 1.1. Where tenacity was not involved the percentage of dead leaves was 66, 47, and 34 for the nonsprayed and the red and yellow types.

Red Leaf Disease of Grapes in California Cured by Controlling Mites. WM. B. HEWITT, H. E. JACOB, E. L. PROEBSTING, and J. F. LAMIMAN. A destructive type of red leaf disease occurs in many vineyards in California. It usually appears by midsummer as a red coloration of the leaf tissue between the primary veins, causes partial to complete defoliation by late summer, followed by a weak development of new terminal leaves. Fruit often fails to mature or shrivels, causing a partial to complete loss. Heavy fertilization with potassium sulphate and trunk injections of manganese, copper, iron, magnesium, uranium, arsenic, and zinc salts failed to prevent red leaf. A spray of 0.25 per cent Selocide (a selenium product) plus 5 lb. of wettable sulphur was applied in July, 1937, to a plot of 114 Zinfandel vines to control the mite, *Tetranychus pacificus.* Mite control was obtained and red-leaf development checked. In 1938 spray applications of Selocide or ammonium polysulphide gave control of red leaf. Applications of selenious acid to pruning wounds failed to control the disease. Another type of red leaf in the same vineyards distinguished by a more intense red; complete vein coloration has failed to respond to the above treatments.

A Transmissible Disease of Grapevines. WM. B. HEWITT. During the past 5 years a destructive disease has become increasingly worse in the vineyards of some of the grape-growing districts of California. Losses have ranged up to 30 per cent of the vines. The symptoms are similar to those of the so-called California vine disease described by Newton B. Pierce in 1892. Symptoms vary somewhat with the varieties. On the variety Emperor the leaves show a dark green veinbanding and a puckering of the tissue between the veins. The vines usually die the season following the first appearance of leaf symptoms. On the variety Ribier, there are no distinct leaf symptoms. The vines wilt and dry up in mid- and late summer, usually with a heavy crop. In early fall most varieties show dying of leaf margins and tissues between the large veins. In late summer some of the canes show a dying back from the tips, and the unkilled portions of the cane mature only in irregular dark brown patches. The fruit often becomes soft or may shrivel and dry up. The disease has been transmitted only by root grafting and by budding.

Internal Bark Necrosis of Delicious Apple, a Physiogenic "Boron-deficiency" Disease. E. M. HILDEBRAND. The work of Berg (1934) and others indicates that "internal bark necrosis" is not a pathogenic disease. Its physiogenic nature was first reported by Young and Winter (1937). The present paper gives confirmatory evidence. In sand culture on full nutrient solution minus boron, Delicious trees showed stunting, rosette, bronzing of terminal leaves, internal bark necrosis, and dieback. This solution plus boron (1 p.m.m.) gave vigorous growth. Less boron (½ p.m.m.) gave abnormal growth in which the shoots were smaller in diameter and so limber as to preclude upright growth. Apple seedlings showed striking boron-deficiency symptoms. The lag in symptom development on McIntosh indicates that its boron-requirement threshold is different from and less exacting than that for Delicious. The presence of diseased Delicious interplants in a healthy McIntosh orchard supports this observation. In preliminary experiments borax applied to the soil in April greatly reduced bark symptoms on young Delicious and, simultaneously, stimulated a more vigorous growth. Boron-deficiency symptoms appear as spherical necrotic areas in the phloem. Meristematic activity is stimulated about the fibers of the pericycle. Hyperplasia is followed by necrosis, the cells dying progressively from the inside out. The affected areas enlarge and usually join neighboring areas, causing elevations on the bark surface, the first external symptom.

Two Fungi (Valsa leucostoma and V. cincta) Besides Brown Rot (Sclerotinia fructicola) Prominently Involved in Peach-Canker Complex. E. M. HILDEBRAND. In recent years measures, ordinarily adequate for brown-rot control, have repeatedly failed in combatting the increasingly troublesome peach-canker complex in northern United States and

Canada. Isolations from cankers collected in New York at monthly intervals from March to November have yielded 3 parasitic fungi. These, named in order of incidence, are *Valsa leucostoma, V. cincta,* and *Sclerotinia fructicola,* their approximate percentages being 57, 40, and 3. All 3 fungi produced cankers at some time during the year by wound inoculations. Brown-rot cankers resulted only between June and October. Conversely, *V. cincta* was apparently infective only during the dormant season from October to May. *V. leucostoma* proved a year-round parasite, and, although less virulent than *V. cincta* during the dormant season, was approximately equally virulent to *S. fructicola* during the growing season. Under natural conditions the more important infection courts were pruning wounds; diebacks; dead bud; fruit spurs and pedicels; mechanical, crotch, winter, borer, and other injuries; leaf scars and lenticels. Cankers produced by the brown-rot fungus commonly heal more readily than the others. This may explain why the *Valsa* fungi are so largely responsible for the perennial type of canker now prevalent in New York.

Delayed Spraying of Tomatoes. J. G. HORSFALL and J. W. HEUBERGER. Quadruplicate tomato plats were sprayed weekly in 1938 with Bordeaux and red cuprous oxide according to 2 schedules: all-season and commencing July 28. By mid-September the percentage defoliation from *Alternaria solani* for the 2 schedules was 36.4 and 30.1 for Bordeaux, 30.4 and 29.5 for red cuprous oxide, and 66.4 for nonsprayed. The disease index based on 5 as a maximum was 1.74 and 2.51 for Bordeaux, 2.36 and 2.94 for red cuprous oxide, and 4.98 for nonsprayed. The yield in tons per acre with its standard error was 6.96 ± 0.29 and 7.59 ± 0.84 for Bordeaux, 9.27 ± 0.60 and 8.39 ± 0.26 for red cuprous oxide and 7.46 ± 1.00 for the nonsprayed. Four sprays during the last third of the season gave essentially equal disease control and yield as 12 sprays during the whole season. Commercial growers reported success with 3 delayed sprays. It did not seem necessary to spray before the appearance of the first leaf yellowed by blight. In New York State it seems that heretofore tomatoes have been sprayed early in the season, when spraying was most injurious and least required, and have not been sprayed late in the season, when spraying was least injurious and most required.

A Maple Blight in Rhode Island. F. L. HOWARD and N. CAROSELLI. A disease of *Acer platanoides, A. pseudo-platanus, A. nigrum, A. rubrum,* and *A. saccharum,* has become prevalent in Rhode Island. The leaves of infected branches are fewer and smaller and abscise earlier than is the case with those of healthy branches. Added to these external symptoms are the successive dying of branches, a reddish ooze or "bleeding" from small fissures in the trunk and scaffold branches, and the ultimate formation of trunk cankers. Internal symptoms are evident as reddish-brown discolored areas, often with olive-green margins, extending from the roots upward to the dying branches. The inner bark, cambium, and from 1 to 10 annual layers of sapwood may be affected in vertical strips varying from 1 to 10 inches wide. Pockets, in which sap accumulates under pressure, are formed at intervals in the sapwood. Isolations from infected sapwood have consistently given a pythiaceous fungus the pathogenicity of which is being tested.

Chemical Control of Nematodes in Tomato Greenhouses. F. L. HOWARD, F. L. STARK, and J. B. SMITH. Control of root-knot nematode, *Heterodera marioni,* by various chemicals was tried in greenhouse soil *in situ.* Chloropicrin and carbon disulphide, applied by direct injection, proved superior to aqueous drenches and compared favorably with steam. Surface watering after injection seemed effective in confining the chemical vapors. Complete eradication of the nematodes was not necessarily the most profitable in tomato production, for dosages of chloropicrin, which delayed initial nematode infection until an extensive fibrous root system had developed, were sufficient to produce normal yields. Other points of interest noted during the experimentation were (1) that indicator crops could be effectively used to forecast subsequent nematode damage, (2) that the amount and type of root development were dependent upon the soil treatment, and (3) that the control of nematodes was not necessarily directly proportional to the fruit yields obtained.

Bordeaux Mixture as a Summer Fungicide for Peaches. R. H. HURT. Four applications of 3 concentrations of Bordeaux mixture (1–3–100, 1½–4½–100, and 2–6–100) applied to 4-year-old peach trees gave excellent control of scab and brown rot without causing injury to either fruit or foliage. It was found that Bordeaux mixture in combination with the zinc-lime spray is safer than Bordeaux alone. The above concentrations of Bordeaux mixture, however, will not prevent arsenical injury on the twigs and leaves of the peach tree. Although Bordeaux at the concentrations used was more efficient in the control of brown rot, it was somewhat objectionable as a pre-harvest spray because it spotted the fruit and had to be removed by brushing.

Removal of Spray Residue with Sodium Hydroxide, Sodium Carbonate, and Acetic Acid. R. H. HURT. A 3-bath treatment, with the first bath containing 15 lb. of sodium hydroxide and 15 lb. of sodium carbonate to 100 gal. of water; the second, 5 to 15 gal. of 28 per cent acetic acid to 100 gal. of water; and the third, plain rinse water, proved very effective in removing heavy residues of lead and arsenic from apples. The higher concentration of acetic acid at 110° F. proved fairly effective in destroying mold spores on the surface of the apples. Newton Pippin, Winesap, Ben Davis, and York Imperial apples were washed with this method without any noticeable injury to the fruit.

Apparent Localization of Phony Disease Virus in the Woody Cylinder. LEE M. HUTCHINS. Numerous attempts have been made to inoculate normal peach trees with phony disease by grafting on their roots patches of bark from roots of phony-affected trees. A high percentage of the patch-bark grafts made successful unions, but in no case was the disease transmitted in this manner. However, as previously reported, when whole root sections consisting of both bark and wood from phony-affected trees were grafted on roots of normal trees, the disease was transmitted in all cases where growth union took place. Therefore, it would appear that the virus of phony disease may be localized in the woody cylinder, although this point has not yet been proved by direct inoculation, using only the woody cylinder for graft inoculum, because growth unions have not been secured in attempted graftage of this nature. In this connection, it is interesting to recall that the chemical laboratory test for phony disease gives a positive reaction only in the wood, the bark giving no reaction. In research on suspected virus diseases of woody plants, it would be advisable to include in the tests graft inoculum consisting of both bark and wood, especially in cases where patch-bark grafts give negative results on transmission.

Promising Results of Heat Treatments for Inactivation of Phony Disease Virus in Dormant Peach Nursery Trees. LEE M. HUTCHINS and JOHN L. RUE. Several series of experiments involving hot water-bath treatments of dormant phony-disease-affected peach nursery trees for inactivation of the causal virus were conducted in 1930–1937, inclusive. The earlier experiments furnished much information on the time-temperature combinations that the trees will survive, but permanent recovery from the disease was not obtained. Based on these results, a series of similar experiments was performed in January, 1937, in which dormant nursery trees, inoculated with phony disease a year previously and 2 years old at time of treatment, were completely immersed in hot water. All trees surviving an immersion of 40 minutes or longer at 48° C. grew normally; and on October 3, 1938, 20 months after treatment, showed no symptoms of phony disease. Such trees gave a negative laboratory test for the disease on the latter date. Trees immersed 35 minutes or less at 48° C. showed typical symptoms of the disease on October 3, 1938, and gave a positive laboratory test. Although apparent inactivation of the virus has occurred from exposure for 40 minutes or more at 48° C., the trees are being retained for further observation.

White Rust of Spinach. S. S. IVANOFF. White rust on spinach appeared in epidemic form during the 1937–1938 season and caused serious damage in southern Texas. The disease appeared in December and spread rapidly until the end of the season, when no healthy plants remained in the fields examined. The disease has been known among growers for several years, but, heretofore, has caused little damage. The symptoms appear as white pustules (sori) scattered mainly on the lower surface of the leaf. Yellowing and mosaic-like coloration of affected leaves also occurred under some conditions. The pathogen probably is *Albugo occidentalis.* The conidia are hyaline. The light to dark-brown oospores are found in abundance within diseased tissue, and their surface is finely and shallowly reticulate, appearing pitted. Conidia and oospores appear indistinguishable from those of the white-rust pathogen affecting *Chenopodium capitatum.* About 150 spinach varieties and strains obtained from various parts of the world were tested for resistance to white rust. No immune plants were found, but differences in susceptibility were apparent. In the commercial fields the commonly grown Bloomsdale Longstanding was more severely affected than Viroflay. Many fields of Viroflay yielded a normal crop, although the plants showed numerous small pustules on the lowermost leaves.

The Relation of Copper Fungicides to Lead Arsenate-Lime and Fixed Nicotine-oil Sprays. K. J. KADOW, M. W. GOODWIN and S. L. HOPPERSTEAD. The compatibility of copper spray materials with lead arsenate-lime and fixed nicotine-oil sprays was studied under field and laboratory conditions. All the insoluble coppers gave slightly better finish to Jonathan, Stayman, and Lily of Kent apples when used with lead arsenate and lime. None of them however, was so effective as Bordeaux mixture in the prevention of arsenical injury. Oxo-Bordeaux, copper hydro, Compound A, and Cupro K gave commercially satisfactory control of arsenical burn. Coposil, Basicop, copper phosphate and Z-O were unsatisfactory in this connection, although in all cases, the injury was less than half as severe as the lead arsenate-lime checks, which lost at least 65 per cent of their leaves by harvest time. Excepting the copper phosphate-lime-bentonite spray, insoluble coppers with lead arsenate-lime reduced both copper and arsenic residues over Bordeaux lead arsenate sprays or lead arsenate-lime alone. Fixed nicotine almost doubled the residue of any particular insoluble copper over lead arsenate-lime. All the copper sprays injured fruit and sometimes leaves, when used with fixed nicotine without lime. Fruit was often unmarketable. Fixed nicotine increased solubility of insoluble coppers from 1 to 25 times. Only Bordeaux, lime and Oxo-Bordeaux release nicotine rapidly, while the other coppers effect its release rather slowly.

Calomel as a Soil Treatment for the Control of Potato Scab in Michigan, Long Island, and New Jersey Soils. GLENN KENKNIGHT. In 1937 scab-infested soil was obtained from Long Island and from New Jersey for comparison with local soil in scab studies. The soils were stored for several weeks in a dry greenhouse and became contaminated with dust from local scab-infested soil before treatments were made. In a pot experiment calomel failed to control scab in any of the 3 soils. In 1938 scabby potatoes as well as scab-infested soil were obtained from Long Island for further pot experiments. Fifty parts per million of calomel in the soil controlled scab in both Long Island and Michigan soils that had been steam-sterilized and infested with *Actinomyces* from Long Island potatoes and soil, and protected from dust. Calomel caused an increase in scabbing of potatoes in both Long Island and Michigan soils infested in like manner with Michigan scab organisms. In all cases the controls were scabby. Calomel controlled Rhizoctonia scurf.

Spraying Experiments for Control of Coccomyces Leaf Spot of Sour Cherry. G. W. KEITT and C. N. CLAYTON. Averaged results from replicated Montmorency plots sprayed at Sturgeon Bay, Wisconsin, in 1938, appear in the following table:

Treatment[a]	Percentage defoliation		Index of average weight per fruit
	Aug. 2–4	Sept. 7–15	
Untreated	98	99	121
Bordeaux, 6–8–100, 1, 2, 3	4	7	100
Bordeaux, 6–8–100, 1, 2	4	10	100
Bordeaux, 6–8–100, 1, 2, 2A, 3	2	4	97
Bordeaux, 3–4–100, 1, 2, 2A and 6–8–100, 3	2	6	99
Bordeaux (high magnesium lime), 3–4–100, 1, 2, 2A and 6–8–100, 3	2	4	104
Lime sulphur, 1–40, 1, 2, 2A, 3	44	66	109
Coposil + lime, 2–4–100, + Orthex, 1–400, 1, 2, 2A, 3	44	79	112
Basi cop + lime, 3–8–100, 1, 2, 2A, 3	24	48	107
Cupro-K + lime, 3–6–100, 1, 2, 2A, 3	67	88	109
Cupro K, 3–100, 1, 2, 2A, 3	24	52	110

[a] Dates of application were: 1, May 26; 2, June 9; 2A, June 23; 3, July 30. Hydrated lime, high calcium unless otherwise stated was used.

Bordeaux mixtures gave superior disease control, but Bordeaux-sprayed fruit was smallest. Dilute Bordeaux gave promising results. Subsequent performance of the trees should be considered in evaluating those results.

Stony Pit, a Transmissible Disease of Pears. J. R. KIENHOLZ. Stony pit is described as a virus disease of pear trees. Gnarled or pitted fruit results at maturity. The tissue at the base of the pits generally becomes necrotic or corky and produces numerous sclerenchyma cells. A "measled" bark condition is associated with the fruit pitting on Bosc. In the advanced stage of the trouble, diseased bark resembles that of a mature oak tree. The bark symptoms may appear first, either on the stock or on the scion, and may not become visible in the other for several years. A faint mottling or a veinlet chlorosis of the leaves appears to be associated with stony pit. Other pear varieties have been observed that show symptoms similar to those of stony pit on Bosc. A decrease in fruit pitting, resulting from treatment with zinc, copper, boron, or manganese, was not established. The disease was readily transmitted to healthy Bosc and Anjou trees from diseased Bosc buds. In most caes the symptoms appeared in the second season. The Bartlett variety was found to be either tolerant or immune by the same method. The disease is known to be present in California, Oregon, and Washington. Control measures have not been studied.

Physiologic Specialization in Fomes lignosus. THOMAS H. KING. Isolates of *Fomes lignosus* from Malaya and Liberia differed strikingly in cultural characters, in growth in certain sugar media and at different hydrogen-ion concentrations, and in their reaction to soil moisture, soil temperature, soil type, and fertilizers. The Liberian isolate is more pathogenic to young plants of *Hevea brasiliensis* than the Malayan isolate. Superphosphate fertilizer stimulated the growth and pathogenic activity of both isolates while ammonium phosphate was inhibitory.

The Occurrence of Lysis in Certain Crosses of Sphacelotheca sorghi. THOMAS LASKARIS. Chlamydospores produced by crosses between certain haploid lines of *Sphacelotheca sorghi* germinated abnormally, producing promycelia that disintegrated either before or after the formation of sporidia or that were very irregular in shape, with swellings and protuberances that finally burst. Chlamydospores from one of these "lethal" crosses were significantly larger than those from normal crosses and produced promycelia and sporidia that averaged 2½ to 3 times the normal size. Some promycelia attained a length of 200 μ but failed to form septa and sporidia. Occasional promycelia resembled somewhat those of the Tilletiaceae. This tendency to autolysis appears to be governed by genetic factors, as it has persisted through 2 chlamydospore generations and is characteristic of certain combinations of lines only.

Some Recent Disease Developments in Forest Tree Nurseries. DENNIS H. LATHAM and W. C. DAVIS. Further evidence has been obtained of the efficiency of crude orthophosphoric acid in decreasing damping off and in stimulating growth of conifers; similar results have been obtained more cheaply by substituting ferrous sulphate for part of the acid. Recent observations have failed to confirm the reported increase in the emergence of black locust associated with post-sowing treatments with formaldehyde. From results on a nursery area treated with large amounts of alkaline sand it appears impracticable to restore such soil to a pH on which pine can be successfully grown by heavy application of sulphur. Preliminary inoculation tests have failed to establish the parasitism of *Sclerotium bataticola* associated with serious losses of conifer seedlings in some nurseries in the Central and Southeastern States. Cedar blight has caused serious losses in a number of nurseries and has been observed on native red-cedar trees; the effectiveness of spraying and roguing for controlling this disease continues dubious. During the current season serious winter-storage losses were experienced with black locust and leaf spot diseases were widespread.

Mycorrhizae and Pseudomycorrhizae on Pines. DENNIS H. LATHAM, K. D. DOAK, and ERNEST WRIGHT. New seedbeds inoculated with similar soil from pine beds in Missouri confirmed the benefit reported from similar inoculations in Australia and elsewhere. Ten cubic yards per acre gave a top growth increase of 70 per cent, whereas 5 cubic yards gave less than half this increase. The majority of cases of poor growth in the United States are apparently not associated with mycorrhizal deficiency. In Indiana a failure that was so associated was more easily corrected by fertilizer than by inoculation. Even in new conifer nurseries in the Prairie States, growth is usually satisfactory without inoculation. It is, nevertheless, perhaps desirable to inoculate nursery soils lacking mycorrhizae, even though growth appears satisfactory, assuming that the planting stock produced would then have mycorrhizae, and that this might give it a better chance to survive on poor planting sites. The most reasonable supposition on the basis of available evidence is that deficiency, or, in some cases, unbalance of mineral nutrients makes the seedling susceptible to invasion by mycorrhizal fungi. Any benefit to the host may be due mainly to preventing the development of pseudomycorrhizae, which, under field conditions, involve most non-mycorrhizal short roots and apparently reduce the absorbing surface and ability to take up mineral nutrients below that of either mycorrhizae or uninfected short roots.

Further Experiments on the Cause of "Purple-top Wilt" of Potatoes. J. G. LEACH. Field experiments have demonstrated that the wilt of potato plants produced in the greenhouse by the tarnished plant bug (*Lygus pratensis*) is not identical with the prevalent "purple-top wilt." Typical symptoms of purple-top wilt were produced in 6 of 8 cages into which were introduced aster leaf hoppers (*Macrosteles divisa*) taken from a variety of plants in nature. Typical symptoms appeared in only 1 cage of 11 in which tarnished plant bugs were reared, and in 2 of 11 cages into which no insects were introduced. These results, together with circumstantial evidence, indicate that the disease may be aster yellows, but further experiments are necessary. Purple-top wilt was not transmitted as such through the tubers; but the tuber progeny of plants affected with the disease were much less vigorous than the progeny of healthy plants. Tubers from plants affected with purple-top wilt were no more subject to spindling sprout than tubers from healthy plants.

Influence of Moisture and Other Factors on the Efficiency and Safety of Sugar-beet-seed Treatment. L. D. LEACH and B. R. HOUSTON. Large scale experiments in central California indicate that damping off of sugar beets usually can be controlled by seed treatment with Ceresan or red oxide of copper. Occasional cases of mercury injury in strip plantings in growers' fields necessitated the identification of the factors involved. Storage of small lots of treated seed in cloth bags, under dry conditions, for 12 months did not reduce germination nor vigor of seedlings. Applications of organic mercury compounds considerably in excess of that necessary for maximum efficiency produced no injury when the seed was planted under ideal conditions. Low moisture content of soils exerted little influence. Saturation of Ceresan-treated seed with water and subsequent storage in air-tight containers for 1 or 3 days produced significant injury. Under such conditions over dosage of the dust increased the hazard. Addition of 10 per cent moisture to treated seed, subsequently stored for 10 days before planting, retarded germination and produced stunted seedlings. Addition of 6 per cent moisture and confinement for 30 days resulted in injury to part of the seedlings, whereas 2 per cent added moisture was non-injurious.

Practical Application of Indexing for Sclerotium rolfsii Infection on Sugar Beets and Some Modifying Conditions. L. D. LEACH and B. R. HOUSTON. Soil samples have been collected from 6,333 acres intended for sugar-beet planting during the past 3 seasons. The samples have been washed through a series of screens, the sclerotia recovered, and their germinability determined by methods previously described. Viable populations determined in this manner provided a basis for predicting the amount of infection a grower was likely to suffer if a sugar-beet crop was subsequently planted. Among 14 fields that yielded no sclerotia, 7 showed no infection on subsequent crops, and 5 others suffered less than 1 per cent loss. Samples from 11 fields showed a population greater than 100 viable sclerotia per square foot and all except 4 showed losses of over 10 per cent. These 4 fields all received heavy applications of nitrogenous fertilizers. Experiments have repeatedly demonstrated that applications of over 100 lb. of nitrogen per acre will reduce the percentage of infection by approximately half. Fields that suffer after planting from high water table, seepage, or over-irrigation without adequate drainage almost invariably show more disease than was anticipated. Likewise, a high percentage of doubles or multiply beets will [illegible] increase the amount of disease.

A Bacterial Wilt of Lespedeza. C. L. LEFEBVRE, T. T. AYERS and H. W. JOHNSON. In the summer of 1937 an apparently hitherto unreported bacterial wilt of annual lespedeza (*Lespedeza stipulacea* and *L. striata*) was observed to be causing considerable defoliation and death of infected plants at Arlington Experiment Farm, Arlington, Va. Subsequently, the causal organism was isolated from diseased plants of annual lespedeza from Missouri, Kansas, Illinois, Tennessee, and New York. The organism first causes dark, water-soaked areas on the leaves, but, later, these become grayish-brown, desiccated, and curled. Eventually, entire plants wilt and die. The stems of the diseased plants frequently crack open and a yellowish bacterial exudate forms and hardens. Similar drops of exudate are sometimes seen on the leaves. Greenhouse inoculations have shown several strains of annual lespedeza to be susceptible to the wilt disease. The susceptibility of perennial species of lespedeza also has been established. The bacterium is a rod-shape, gram negative, monotrichous organism. Colonies on agar are yellow, and those on nutrient agar are extremely viscid. The organism liquefies gelatin, egg albumin, and blood serum, and forms both hydrogen sulphide and indol. Neither acid nor gas are formed from sugars. Investigations have not progressed far enough to prove definitely whether the lespedeza organism is an undescribed species.

Hyperauxony of Nodules of Phaseolus vulgaris.[1] GEORGE K. K. LINK and VIRGINIA EGGERS. Using Van Overbeek's method for determining the auxin content of plant tissues and for differentiating auxins, it was found that ether extracts of the nodules have a decidedly higher auxin content than the roots that bear them. Heating the extracts with HCl and NaOH, respectively, at 100° C. indicates that the extract of bean roots contains one or more auxins that behave like auxentriolic acid, and also one or more that behave like indole(3)acetic acid. One or more auxins of both types occur in extracts of nodules, the latter type, however, accounting for most of the auxinic activity of nodule extracts. These findings indicate that *Phaseolus vulgaris* contains auxin *a* or a closely related auxin; that its nodule is characterized by a hyper-auxony that which in part, at least is attributable to an increase of one or more auto-auxones of the bean root and hetero-auxones of the indole(3)acetic acid type formed by *Rhizobium phaseoli* in tryptophane containing media.

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Production of Growth Substance on Peptone Broth by Crown-gall Bacteria and Related Non-gall-forming Organisms. S. B. Locke, A. J. Riker, and B. M. Duggar. The production of growth substance in peptone broth by crown-gall bacteria, an attenuated sister culture, and an avirulent organism (*Bacillus radiobacter*) has been studied with Went's Avena technique. The non-concentrated culture fluids were tested at frequent successive intervals during 3 weeks. Five tests consistently showed that all 3 cultures produced growth substance in approximately equal amounts. Differences were not significant. In a representative trial the maximum concentration, equivalent to 130 gamma beta-indole-acetic acid per liter of culture fluid, was reached at the end of 21 days. The growth substance present in all 3 cultures was more stable in alkaline than in acid solution, and was closely related to, if not actually, beta-indole-acetic acid resulting from bacterial acid on tryptophane or other amino-acids present. Since the virulent and attenuated crown-gall bacteria grew at similar rates, when inoculated into tomato, but differed strikingly in their ability to induce pathological growth, it was concluded that pathogenicity may be dependent primarily on some character aside from the production of beta-indole-acetic acid.

An Analysis of Factors Causing Variations in Spore Germination Tests of Fungicides. S. E. A. McCallan and Frank Wilcoxon. The variation in germination was studied for spores of *Sclerotinia fructicola* on glass slides in the presence of various copper sprays and copper sulphate. Factors held constant were temperature of spore production and germination, age of culture, medium, spore concentration and orange-juice stimulant. The limiting factor in fungicide comparisons was the difference in behavior of the same fungicide in experiments replicated at different times. This variation was not attributable to different batches, age or amount of agar in the tubes, size of tubes, tightness of cotton plugs, spores from different field isolations, or from mass or single spore cultures. An important source of variation is the unavoidable use of different transfers for the different experiments. The replicate-transfer variation may be reduced by modifications in the technique of obtaining the spores, especially by centrifuging to remove substances dissolved unevenly from the agar. Tests in which 7 insoluble copper fungicides at 4 concentrations were replicated 8 times showed a high variation between different experiments, of which a portion could be ascribed to the use of different transfers. For adequate comparisons, spores from several different transfers should be used in each experiment and the experiments replicated several times.

Some Further Experiments with Seed Disinfection in Cereals. J. E. Machacek and F. J. Greaney. The results of several years' field tests showed that organic mercury dust preparations were superior to other seed disinfectants for the control of seedling blight and root rot of wheat in Manitoba. As far as increases in yield were concerned, seed disinfection for the control of these diseases was profitable only when either the seed or the soil was severely infested by pathogenic fungi. These findings suggested that recommendations for seed treatment should be based on actual proof of such infestation. While soil fertilization increased the yields from nontreated wheat seed, as shown by a 3-year test at Winnipeg, no additional increase in yield was secured by treating the seed. In fact, in all of these trials, seed treated with organic mercury dusts yielded slightly less than nontreated seed, regardless of the presence or absence of soil fertilizer. The failure of the mercury dusts to increase the yield was accounted for by the fact that severely infested seed was not used in these trials, a result that emphasized the importance of using severely infested seed when evaluating the merits of fungicides under ordinary field conditions.

Comparative Studies on Two Genotypes of Nicotiana tabacum Resistant to Nicotiana Virus 1. H. H. McKinney. Inoculations of Ambalema and 448A plants when young with *Nicotiana Virus 1* show that the spread and increase of the virus is less in 448A. At time of flowering all leaves of Ambalema contained virus, whereas the upper leaves and leaves of upper side-shoots of 448A remained virus-free. Except for a few top immature leaves which apparently were highly resistant or possibly immune the virus-free leaves of 448A were susceptible as virus increased in them when inoculated. All leaves longer than three inches on healthy Ambalema and 448A plants approaching flowering were inoculated. Subsequent assays indicated less virus increase in upper than in lower leaves, the upper leaves of 448A showing the least. The major increase of virus in 448A during the systemic period apparently is confined to relatively few scattered cells or small groups of cells which become less numerous in subsequent leaves as virus translocation decreases. Genotype 448A is very susceptible to several of the cucumber and potato viruses, and it reacts differently to some of the mutants of *Nicotiana Virus 1* than it does to *Nicotiana Virus 1*.

Invasiveness of Phytomonas stewarti in Sweet Corn Supplied with Different Amounts of Nitrogen. GEORGE L. MCNEW and ERNEST L. SPENCER. Sweet-corn seedlings of the variety Golden Bantam, grown in sand cultures and supplied with a nitrogen-deficient nutrient solution, were less severely wilted by *Phytomonas stewarti* than similar seedlings supplied with nitrogen. Studies were undertaken to determine whether the nitrogen content of the tracheal sap had any direct effect in regulating the invasiveness of the bacteria. Exudates from the tracheal tubes of plants receiving different amounts of NH_4NO_3 wre collected and incorporated into a dextrose-agar medium. The growth of the bacteria on these media was closely correlated with the nitrogen content of the exudates. Little or no growth was obtained on media containing the exudate from nitrogen-deficient plants. Virulent cultures apparently grew so poorly in nitrogen-deficient seedlings that they were sometimes replaced by less pathogenic variants after a series of host passages. Similar changes were not observd in comparable seedlings supplied with NH_4NO_3. These tests indicate that nitrogen-deficient seedlings are not severely wilted by *P. stewarti* because the highly virulent strains of the bacterium make only a limited growth in the tracheal tubes and may even be replaced, in time, by less virulent strains.

X-Ray Diffraction Study of Tobacco Mosaic Virus Proteins Prepared by the Sodium Sulphate Method. D. K. MCREYNOLDS, N. S. GINGRICH, and CARL G. VINSON. The material was placed in a flat cell of approximately optimum thickness with faces of exceedingly thin-blown glass. This cell was placed in a gas-tight camera having 15.41 cm. as the perpendicular distance from the flat photographic film to the cell. Helium was passed through the camera to reduce gas scattering. Approximately monochromatic copper Kα radiation was secured through the use of a copper target tube and a thin nickel filter before the apertures for collimating the X-ray beam. Exposures were about 100 hours. The Bragg spacings, d, ranged from 2.43 A to 14.3 A, though spacing as large as about 50 A could have been detected. The correlation of these spacings with those obtained by Wyckoff and Corey is not very good. In particular, the very large spacings are absent in the present material, and, where practical coincidence is observed, the relative intensities of the lines are not comparable. Wyckoff and Corey do not record spacings less than [illegible].39 A, whereas in the present work, a spacing of 2.43 A is recorded. It is concluded, therefore, that this crystalline-like fraction probably has a lower molecular weight than those reported by Wyckoff and Corey.

Pathogenicity of Actinomycete Isolates on Sweet Potato. W. J. MARTIN and L. H. PERSON. Preliminary tests of the pathogenicity of numerous isolates obtained from pox-infested sweet potatoes were made by placing agar blocks of the isolated organisms on rooted stem cuttings of sweet potatoes in agar plates. Isolates that proved to be pathogenic were further tested in laboratory moist chambers by inoculation of mature potatoes. Positive results were obtained by this method. Sweet-potato plants were then cultured in the greenhouse in 2-gallon crocks of sterilized soil inoculated with isolates of proved pathogenicity. The plants in inoculated soil were stunted and typical of plants in naturally-infested fields. The potatoes when harvested were covered with typical pox lesions. In further experiments plants were grown in 6-inch pots of sterilized soil inoculated with pathogenic isolates. The pots were left in the greenhouse for a while and then transferred to the field, where the plants were allowed to grow. They remained stunted, became yellow to bronze, and some of them died, reactions typical of plants in naturally-infested fields. The isolates were further tested by direct field inoculation, and typical pox symptoms developed from these inoculations also.

A Disease of Gloxinia Caused by Phytophthora cryptogea. JOHN T. MIDDLETON and C. M. TUCKER. A disease affecting the corm (commonly known in commercial fields as the tuber), stem, and leaves of *Gloxinia* (*Sinningia speciosa*) was observed on greenhouse-grown plants in California. In naturally infected plants the leaves are water-soaked, dark brown, and flaccid. Infection progresses from the lamina to the petiole, and, in severe cases, the stem may be attacked. Quite often stem infection is noted in the absence of leaf infection. Symptoms of the disease on the stem are sunken, watersoaked lesions, which may be rather narrow and vertically disposed, or may be rather large and encompass the stem. Badily infected plants collapse and die. Infected corms exhibit soft, sunken, surface lesions. Severely diseased corms usually have dark brown, soft, internal necrotic areas, 1 to 8 mm. in diameter, erratically disposed throughout the underground storage organ. These infected regions may or may not be directly associated with the more common surface lesions. The causal organism is *Phytophthora cryptogea* Pethy. and Laff. The pathogen has been isolated from leaves, stems, and corms of infected *Gloxinia* plants. The various isolates have proved pathogenic to these plant parts on inoculation. This disease, apparently identical with a Gloxinia disease reported at various times from Europe, has not been previously reported from this country.

Control of Cercospora Leaf Spot of Peanut with Copper and Sulphur Fungicides. LAWRENCE I. MILLER, E. T. BATTEN, and S. A. WINGARD. Results of experiments conducted in Southeastern Virginia indicate that Cercospora leaf spot of peanut can be satisfactorily controlled with copper and sulphur fungicides. Three applications of Bordeaux mixture proved effective in controlling leaf spot and caused no injury to the plants. Liquid lime-sulphur sprays gave satisfactory leaf-spot control, but caused a burning of the foliage and stunted the plants. Wettable-sulphur sprays caused no injury but failed to control leaf spot. Certain proprietary sulphur materials, applied as dust, controlled leaf spot and caused no injury to the plants. Leaf-spot control resulted in an increased yield of hay and nuts.

Apple Rusts in Relation to Varietal Susceptibility. PAUL L. MILLER. It is now known that in central and eastern United States there are 3 species of *Gymnosporangium*, namely; *G. juniperi-virginianae* Schw., *G. globosum* Farl., and *G. clavipes* C. & P., that may cause loss in apple orchards, instead of one as was generally assumed at the time these studies were begun. Evidence is presented to show that the failure to recognize and distinguish these rust diseases may account for the inconsistent records of apple varietal susceptibility.

Snapdragons Resistant to Two Races of Puccinia antirrhini. RAY NELSON. In the 1929 floricultural trial gardens at East Lansing all snapdragon plants except one were killed by rust. Segregation in the snapdragon progeny for color and resistance proved its heterozygous constitution. The progenies of selections made in 1931 and subsequent years were inoculated with rust and planted out-of-doors under conditions favorable for rust development. By repeated selection and inbreeding a number of lines suitable for bedding have been stabilized for color and resistance to the form of rust prevalent in Michigan. Plantings of these varieties at East Lansing and Kalamazoo in 1938 were inoculated, respectively, with races 1 and 2 of *Puccinia antirrhini.* Selections resistant to race 1 were either susceptible, partially resistant, or highly resistant to race 2. Commercial varieties resistant to or immune from race 1 were very susceptible to race 2. Ordinary commercial varieties were killed by mid-summer. Complete susceptibility to race 2 of commmercial varieties resistant to race 1 and resistance to race 2 of some descendants of the 1929 selection indicate that these selections are genetically different from commercial rust-resistant varieties.

Progress in Control of Onion Mildew (Peronospora destructor) in New York. A. G. NEWHALL. Spraying 4 times at weekly intervals again failed to hold mildew in check even though 600 to 900 gal. per acre application of potash-rosin-lime sulphur, red copper oxide, and malachite green were used with a number of wetting agents such as "Grasselli Spreader," "Ultrawet," "Santomerse," cottonseed oil emulsion, "Lethane Spreader," etc. The large amounts of new leaf growth occurring between applications (10 to 15 in.) may have contributed to the failure. Control by eradication of the many garden plantings of diseased topset and other perennial onions was attempted on a township-wide basis. Over 80 such plantings were voluntarily removed in early May from Marion township, comprising 30 square miles of Wayne County. Forty-seven of these were found in a total of 344 farm gardens and 33 in the village of Marion (population 900). Over 60 per cent were harboring mildew. This cleanup in May apparently resulted in a reduction in the percentage of diseased commercial onion fields from 68 per cent outside the area down to 28 per cent inside, when examined on August 26. Local spot surveys have supported the contention to date that every county has hundreds of these diseased topset plantings.

Two New Electrical Devices for Pasteurizing Soil. A. G. NEWHALL. Flats of soil have been successfully steam heated for the control of damping off when placed for 2 hours on shelves in a dairy utensil sterilizer built along the lines of an Arnold sterilizer with a 1500 watt electric stove element in the base of a tight well insulated box. A pan of water placed on the heater is boiled nearly dry. The steam thus generated and condensing in the soil raises the latter to a fairly uniform temperature of 60° C. with the expenditure of but 1.3 kw. hr. of current per cu. ft. of soil, per 1.3 qt. of water vaporized. For treating soil in benches and ground beds an inverted pan-type of electric steamer has been built of insulating board, with 4 1500-watt strap heaters suspended under long troughs of water inside the pan. The water on boiling off condenses in the soil beneath the pan. With the aid of thermocouples it has been found that heating of the first 9 in. to a pasteurizing temperature occurs in a few hours with the expenditure of approximately 1.5 kw. hr. of current per sq. ft. of soil treated. Tests of ordinary loam have shown that an end-point temperature of 60° C. is sufficient to control damping off, nematodes, and most weed seeds.

Adherence Properties of Copper Fungicides as Determined by Chemical Analyses and by Cataphoresis. A. A. NIKITIN. A study has been made on the effect of oxides of the alkaline earths, zinc and aluminum, on the adherence properties of the insoluble copper fungicides. One method of study involves chemical analyses to determine the amount of residue left on glass plates sprayed with fungicides, dried, and then subjected to artificial rain for a definite period of time. Another method of approach is the use of electrodialysis. The deposit of copper fungicide on the electrode is analyzed to determine the amount of copper. By the use of both methods, each of which is theoretically different, a confirmation was obtained on the adherence of the copper fungicides metal oxide combination. It has been found that in the presence of lime or barium oxide the adherence of copper fungicides is much less than with dolomitic lime, zinc oxide, or aluminum oxide. It should be emphasized that the efficiency of the most commonly used spreaders and stickers is greatly improved when dolomitic lime, zinc oxide, or aluminum oxide is used as a substitute for high-calcium lime.

Chloropicrin as a Seed Disinfectant for Control of Black Rot of Kale. T. J. NUGENT and HAROLD T. COOK. Since the treatment of crucifer seed with mercuric chloride for the control of black rot requires considerable labor and time, as well as special care in handling the seed after treatment, preliminary tests have been made with chloropicrin to determine its value as a seed disinfectant for the control of this disease of kale. Treatment of the seed for 24 hours with chloropicrin at the rates of .88 cc., 1.76 cc., and 3.52 cc. per liter of space, disinfected the seed equally as well as mercuric chloride. The amount of chloropicrin required varied, depending on the amount of seed in the fumigating chamber, the larger quantities being required when the chamber was full or nearly full of seed. This indicates that some of the gas may be absorbed by the seed. Treatment at temperatures between 13° and 40° C. resulted in no apparent difference in its effectiveness as a disinfectant, or in its effect on seed germination. Treatment did not affect the germination of air-dried seed.

Seed Treatment for the Control of Bacterial Blight of Beans. L. H. PERSON and C. W. EDGERTON. For the past 25 years numerous seed-treatment tests, using various dusts and liquids, have been made to determine their effectiveness for the control of bacterial blight of beans, *Bacterium phaseoli* and *B. medicaginis* var. *phaseolicola.* Results have been somewhat variable from year to year, but in no test did the dust treatments give any indication of control, so they were discarded in favor of the liquid treatments. In the spring of 1938, very promising results were obtained by treating the seed from 12 to 14 minutes in a solution of 1 to 500 $HgCl_2$ in 70 per cent ethyl alcohol + 2 per cent acetic acid. Three plantings were made from 7 to 15 different seed lots. Effective control of initial infection on the primary leaves was obtained in all tests. In only one instance was there evidence of a single light infection center in the treated plots, while in the controls, numerous severe infection centers were present in several of the most severely infected seed lots. The treatment causes some reduction in germination.

Bacterial Leaf Spot of Dieffenbachia. P. P. PIRONE. A new bacterial disease of the foliage plant, *Dieffenbachia picta,* is prevalent in several New Jersey greenhouses. Infection is confined primarily to the leaves, where circular, reddish-brown spots (up to 1 cm. in diameter), surrounded by light yellow, water-soaked margins, are produced. As the spots enlarge or coalesce, the centers become brown and dry, and often the area between the spot and leaf margin turns yellow and soon dies. A bacterial ooze usually occurs in the spots on the lower leaf surface, which eventually dries into a thin wax-like layer that peels off readily. A species of bacterium has been isolated consistently from young spots. Typical symptoms were produced in from 1 to 2 weeks by atomizing a suspension of the bacterium on the lower leaf surfaces. The same organism has been reisolated repeatedly from such spots on more than 30 leaves in 3 series of tests. Under commercial conditions the disease is most destructive in late summer and early fall in houses where high humidities prevail, or where the plants are overcrowded, or where considerable overhead watering is practiced. Removal of heavily spotted plants, wider spacing, and the avoidance of overhead watering have resulted in satisfactory control under commercial conditions.

Cercospora Leaf Spot of Strawberry. A. G. PLAKIDAS. In the past three years an apparently undescribed leaf spot, caused by a species of *Cercospora,* has been found on both wild and cultivated strawberries in Louisiana. The spots resemble those caused by *Mycosphaerella fragariae,* but are smaller and somewhat angular. The older spots have white centers, dotted with black specks—the sporophores of the fungus. Both the sporophores and the spores are very long, the former averaging about 130.0 × 5.0 μ, and the latter about 70.0 × 4.0 μ in size. The spores are hyaline, tapering, mostly curved, 1–9 septate. It is evident from the large size of the spores that the Louisiana fungus is different from *C. vexans* C. Mass., reported on strawberries from Italy and Wisconsin. The spores of the Italian fungus are described as 15–18 × 2.3 μ, one-septate, catenulate, and those of the Wisconsin fungus as 6–12 × 2–3 μ, catenulate. Also, the sporophores of the Louisiana fungus are approximately twice as long as those of *C. vexans.*

A New Mycosphaerella Leaf Spot of Strawberry. A. G. PLAKIDAS. A leaf spot of strawberry, caused by an apparently undescribed species of *Mycosphaerella,* has recently been found in Louisiana. The symptoms of this disease are so similar to those of scorch (*Diplocarpon earliana*) that the two diseases are easily confused. The spots are purplish, never developing the white or ashen-gray centers characteristic of the common leaf spot (*M. fragariae*). When many spots coalesce, large, irregular reddish-purple areas develop, imparting to the leaf an autumnal coloration resembling frost injury, and the leaf finally dries up. Mycelium occurs in great abundance in the tissue of the discolored areas. The disease has been repeatedly produced by inoculating either with mycelium or with ascospores. A conidial stage of the causative fungus has not been found. Viable perithecia, typical of the genus *Mycosphaerella,* are produced in great abundance on the dead leaves, both in winter and summer, and a limited number of ascospores have been produced in culture. Numerous mono-ascospore isolations have been made, but in no case were conidia produced in culture. This indicates that there is no connection between this fungus and *Ramularia tulasnei.*

Comparison of Thermal Inactivation Rates of Two Plant Viruses. W. C PRICE. The thermal inactivation rate for tobacco-necrosis virus in freshly extracted juice of diseased tobacco plants has been determined and compared with that found for tobacco-mosaic virus. Although the two viruses have about the same thermal death point, tobacco-necrosis virus is more quickly inactivated at temperatures below 92° C., while tobacco-mosaic virus is more quickly inactivated at temperatures above 92° C. The temperature coefficient of inactivation, Q_{10}, for tobacco-necrosis virus is about 4; that for tobacco-mosaic virus is of the order of 750.

A Rapid Reagent-indicator Method for the Detection of the Mosaic Virus Agent in the Tobacco Plant. AGNES J. QUIRK. The method consists of preparing reagent indicator-tobacco mixtures and linking a definite color reaction in the mixture to healthy and mosaic virus-diseased tissue juices. The method for detecting the mosaic virus agent parallels that of the pH method for the detection of the hydrogen-ion concentration in plant tissues and in culture mediums.

A Microchemical Study of Gum Pocket Formation in Sweet Cherry Wood. T. E. RAWLINS. The gum pockets frequently found in the wood of cherry trees grown in an unfavorable environment originate in the phloem. A new cambium is then formed outside of the phloem gum pockets; this cambium produces new wood cells and the gum pockets thus become imbedded within the wood. Some workers have assumed that the gum pockets in cherry wood arise from the decomposition of wood cells. Since cherry wood contains considerable xylan they were unable to explain why the cherry gum contains much more araban than xylan. It is probable that the araban in the cherry gum is derived from the unlignified phloem walls, while presumably contain considerable araban but little xylan.

Recent Findings Regarding the buckskin Disease of Cherries. T. E. RAWLINS. Healthy sweet cherry scions were grafted on the scaffold limbs of Mahaleb seedlings. A buckskin sweet-cherry scion was placed on one limb of each of the seedlings in 1933. Up to the present time the Mahaleb seedlings and the healthy sweet-cherry scions have not shown any recognized symptoms of the disease. These results suggest that the virus does not pass through Mahaleb to sweet cherry scions and that the disease will, therefore, cause less injury in trees worked in the scaffold limbs of Mahaleb than in trees having a single union with Mahaleb near the surface of the soil. In some orchards the disease has been satisfactorily controlled by removing the diseased trees; in others this treatment has not given satisfactory control. Infected trees may be quickly killed by pouring a 10 per cent solution of sodium arsenite into holes bored tangentially into the sap wood. Attempts to locate an insect vector have been unsuccessful. The insect population on a tree may be determined by spraying with a pyrethrum-oil preparation and collecting the insects on cloth spread under the tree. Rare species may be detected by this method.

The Influence of Crown Gall and Hairy Root on Growth of Young Apple Trees. A. J. RIKER. Measurements have been made on the growth of Yellow Transparent apple trees infected for 7 years with crown gall and hairy root bacteria. The trees were planted as grafts in 1930 in a Kansas nursery, inoculated early in 1931 just above the union with known pure cultures, transplanted in the spring of 1932 to an experimental orchard in Wisconsin, and measured in 1938. There were a total of 161 trees. No significant difference was found in the mortality of the hairy root, crown gall, and nontreated control trees as a result of transplanting or later. The average height was: For crown gall—11.0 ft., for hairy root—8.6 ft., and for uninfected—11.2 ft. (significant difference, 1.27 ft.). The average circumference was: For crown gall—8.8 in., for hairy root 6.4 in., and for infected 9.5 in. (significant difference, 0.96 in.). Sixty-nine trees were removed in 1938. There was only occasional recovery from crown gall and hairy root and only occasional infection of trees smooth when planted. The crown gall and hairy root overgrowths suggested open infection courts for heart-rot organisms, but no heart rot has thus far appeared.

Factors Affecting the Longevity of Urediospores of Puccinia coronata avenae. H. R. ROSEN and L. M. WEETMAN. Oat leaves heavily infected with the uredial stage of crown rust were gathered in June of 2 successive years and kept at various combinations of temperature and relative humidity, with a range in temperature from 5° to 40° C. and in relative humidity from 0 to 90 per cent. Under outdoor (uncontrolled) conditions the spores died in short order; likewise, under outdoor temperatures but controlled humidities, they were short lived. The combination of temperatures and relative humidities in which the spores remained viable and infectious for nearly a year were the following: 5 to 10° C. and 25 to 50 per cent relative humidity. At 15° C. and 25 per cent relative humidity a small percentage of spores were viable for almost a year, the diminution in viability being considerable after 6 months, while at 15° C. and 50 per cent relative humidity the spores retained viability for 6 months but none showed germination toward the end of a year. At temperatures higher than 15° C. and at all humidities utilized, the spores were short lived. The evidence indicates that crown rust urediospores on dead oat leaves play no part in carrying the pathogen from one year to another in Arkansas or in any large area in the United States.

A Non-transmissible Spindling Sprout of Potato. E. S. SCHULTZ. In December, 1937, spindling-sprout Bliss Triumph tubers, grown in certain north central potato localities, were planted in the greenhouse at 60° to 70° F., Beltsville, Maryland. The sprouts on these tubers were so weak and spindling that "hair sprout" clearly describes them. On March 14, 1938, when the plants were harvested, they had developed slender shoots having a diameter about half that of normal plants, and small tubers varying from 10 to 30 g. On July 14, 1938, these young tubers were planted in a greenhouse, Presque Isle, Maine, where they formed stocky, vigorous shoots and developed tubers weighing 120 to 180 g. by October 1. These observations confirm similar results obtained in 1935 and 1936 with spindling-sprout Green Mountain tubers that matured in the field during the summer at Beltsville, but recovered and developed normal plants when grown at Presque Isle. Grafting healthy tubers with plugs from spindling-sprout tubers did not transmit spindling sprout. These observations suggest that unfavorable environmental conditions adversely affected normal functioning of the potato. This non-transmissible type of spindling sprout, though similar in appearance to leafroll and witches' broom, must not be confused with these transmissible diseases.

Effects of Different Dates of Transplanting Tobacco on the Control of Losses Caused by Heterodera marioni. K. J. SHAW. Observation over a period of years by research workers has led to the belief that the time of transplanting tobacco has a definite relation to the severity of root knot caused by *Heterodera marioni.* In order to obtain specific data, experiments on this problem were conducted during the summers of 1936, 1937, and 1938 at the McCullers Tobacco Station, located 10 miles south of Raleigh, North Carolina. Three dates of transplanting (early, medium, and late) were used. No consistent difference was found in the percentage of severe root knot. The reason for this is not clear, although the results indicate that the severity of root knot is correlated with the rapidity of the plants' growth during their early life in the field. For the 3 year averages there was a significant difference in yield and value per acre. The early transplanting yielded 980 lb., valued at $188.52 per acre; the medium 845 lb., valued at $151.91; and the late 732 lb., valued at $122.71.

Variability in Fusarium vasinfectum. C. D. SHERBAKOFF. Results of studies of *Fusarium vasinfectum,* with reference to the possible existence of specialized forms are here reported. During the past 3 seasons over 3500 specimens of cotton wilt were culturally examined. The specimens were obtaind from 11 cotton-growing States. Most of the specimens yielded a *F. vasinfectum*-like fungus, which showed great variation in color, sclerotia, rate of growth, and proportion of micro- and macroconidia, with noticeable difference in morphology of the conidia. The preliminary test of pathogenicity of 7 cultures, selected on the basis of differences in appearance and morphology, indicates that they probably differ markedly also in their virulence and ability to affect different varieties of cotton. (Cooperative project between Division of Cotton and Other Fiber Crops and Diseases and the Tennessee Agricultural Experiment Station.)

Field Survey of the Relation of Susceptible Weeds to Granville-wilt Control. T. E. SMITH and R. K. GODFREY. In field plot experiments, crop rotation consistently provides control of Granville wilt of tobacco (*Bacterium solanacearum*). In the hands of the farmers the results have been variable. Eight species of field weeds known to be susceptible to the disease by natural infection were found growing on fields being rotated for wilt control. Their relation to the problem of field control has been studied on 29 fields during the past 3 years. The population of susceptible weeds was determined during the season immediately before the return of the fields to tobacco in the rotation cycle. No positive relation was found between the population of susceptible weeds in the cultivated crops and the severity of wilt the following year. On certain fields where susceptible weeds were absent or present in only small numbers in the preceding crop, wilt was severe on tobacco. On other fields having the same crop history and a dense population of susceptible weeds in the preceding crop, good control was obtained. The results indicate that the presence of a small number of susceptible weeds in an otherwise immune or resistant growth is not a great disease hazard. Other factors may be responsible for the failure of crop rotation to give consistent control under farm conditions.

The Effect of Nitrogen Nutrition on Concentration of Tobacco-mosaic Virus. ERNEST L. SPENCER. Investigation of the relationship between host nutrition and host response to tobacco-mosaic virus has been continued with a study of the virus concentration in diseased plants supplied with various amounts of nitrogen. Seedlings of Turkish tobacco, *Nicotiana tabacum*, inoculated 3 days after transplanting, were grown in sand cultures and supplied with 3 nutrient solutions containing different levels of nitrogen. At intervals, representative plants in each treatment were harvested, frozen, and subsequently minced. The concentrations of virus in juices expressed from the pulp were measured by the number of local lesions produced on *Phaseolus vulgaris* var. Early Golden Cluster. The concentration of virus in the expressed juice increased as the amounts of nitrogen supplied to the plants increased. Juice from plants dwarfed by an excess of nitrogen had a higher virus concentration than juice from plants receiving a nitrogen level more conducive to rapid growth. From this it is concluded that the concentration of virus in expressed juice is not necessarily determined by the growth rate of the host.

Observations on Stem-rust Epidemiology in Mexico. E. C. STAKMAN, W. L. POPHAM and ROBERT C. CASSELL. The uredial stage of *Puccinia graminis* survived the winter of 1937–1938 in occasional early-sown winter wheat fields in Southern Mexico and in relatively fewer fields in Northern Mexico. This stage apparently can persist on winter and summer wheat throughout the year in Southern Mexico, independently of inoculum from the north; but in Northern Mexico early-sown fields of winter wheat often become infected with spores blown from the north. Supporting evidence is the fact that only 3 physiologic races were found in Southern Mexico, as follows: Race 59, 58 per cent of isolates; race 38, 37 per cent; and race 24, one collection. In Northern Mexico, 9 races were found: Race 38, 42.5 per cent; 49 and 56, 15 per cent each; 17 and 19, 7.5 per cent each; 36, 5 per cent; 11, 32, and 59, 2.5 per cent each. Race 56, by far the most prevalent in the United States in recent years, was found in Northern Mexico but not in Southern Mexico. Further evidence of independent development of rust in Southern Mexico is the resistance of Marquis wheat, attributable to the predominance of races 59 and 38.

(Cooperative investigations, U. S. Department of Agriculture and the Minnesota Agricultural Experiment Station.)

Influence of Environment, after Seedling Emergence, on Loose Smut of Oats and Covered Smut of Barley. V. F. TAPKE. The seedling-infecting smuts of small grains and sorghum invade their hosts during seedling growth from seed to soil surface. Soil conditions during this period, therefore, have been considered the important environmental factors affecting infection and incidence of smut. Recent studies of oats loose smut and barley covered smut, indicate that environmental conditions after seedlings emerge may influence markedly the incidence of smut. Winter barleys and winter oats were grown outdoors and in a greenhouse after the seedlings emerged in autumn. When preemergence conditions included not only favorable soil factors but also favorable position of inoculum beneath seed hulls, deep seeding, high susceptibility of host and other factors, high and similar percentages of smut occurred under both the outdoor and greenhouse environments. When all preemergence conditions were not ideal for infection of the seedlings, as frequently happens in field culture, the different environments after emergence effected striking differences. Under outdoor conditions at Arlington, Virginia, a low incidence of smut occurred. Under greenhouse conditions continuously after emergence or for only a month followed by transfer of plants outdoors, the incidence of smut was high. Evidently, the rugged outdoor environment sustained and the temperate greenhouse environment ameliorated the effects of the relatively unfavorable preemergence conditions.

Effects of Ceratostomella ulmi on Ulmus americana and Some Types of European Elm. JAMES M. WALTER. American elm seedlings and 4 types of European elms were heavily inoculated with *Ceratostomella ulmi* at Oxford, England, in 1937 and 1938. The trees were about 6 ft. tall when transplanted early in 1936. The European types tested were: *Ulmus montana* seedlings, diverse seedlings with characteristics of *U. minor* predominant, Wheatley elm grafted on *U. montana* rootstocks, a vegetatively propagated type, advisedly called X, *U. hollandica*. In 1937, inoculations involved 320 trees of each population except the last-named, which was represented by 200. The severity of wilt symptoms of each tree was evaluated on a scale of 0 to 5, according to proportion of leaves lost prematurely. Average final 1937 severity ratings for the types in order mentioned were: 4.8, 4.0, 3.2, 2.3, 3.0. Trees killed in 1937 were: *U. americana,* 45; *U. montana,* 5; *U. minor,* 7; other types, none. The proportions of leader-dieback, calculated from height measurements on each tree, more clearly showed the greater relative susceptibility of American elm. During 1938 surviving trees of each type were again heavily inoculated and results agreed in general with those for 1937. However, 215 American elms, severely diseased in 1937, were not reinoculated and only 16 of them showed recurrence of symptoms.

A Water-culture Infection Method Used in the Study of Fusarium Wilt of Cotton. R. WEINDLING and G. M. ARMSTRONG. A simple technique has been devised for inoculating cotton plants in water culture with *Fusarium vasinfectum.* The roots of seedlings were submerged for a short time in a nutrient-solution culture of the pathogen, so that a large number of filaments and spores adhered to the roots. A very high percentage of infection was obtained within 6 to 7 weeks, under greenhouse conditions. Equally severe infections frequently occur locally in the field. *Fusarium* isolates, exhibiting different degrees of virulence in soil experiments, gave similar results in the water-culture-infection tests. Water-culture data on the relative wilt resistance of 7 cotton varieties agreed, in general, with field experience. The technique may supplement other methods; (1) as a rapid test procedure in breeding plants for resistance to wilt diseases, and (2) in research upon the course of infection and nature of resistance with plants subject to diseases caused by soil-borne organisms.

Mercuric Oxide as a Soil Antiseptic Against Fusarium Rot of Narcissus Blubs. FREEMAN WEISS and FRANK A. HAASIS. The predominant source of Fusarium bulb rot is the planting of contaminated bulbs. Since fall planting is practiced and subminimal temperatures for progress of rot prevail during winter, conspicuous development of rot is delayed until spring emergence or until harvesting in midsummer. Control of this source of infection requires antiseptic treatment of bulbs; soil fungicides are ineffective. With close planting and favorable environmental conditions, infection of healthy bulbs from proximity to contaminated bulbs and from infested soil may occur. Soil fungicides have prospective value for arresting such spread. Tests with various materials, including copper carbonate, formaldehyde, tetrachlorethane, organic, and inorganic mercuries, indicated the superiority of HgO. $Al_2(SO_4)_3$ increases its effectiveness. In Long Island, with soil initially at pH 4.6, HgO at 40 lb. per acre and $Al_2(SO_4)_3$ at 100–1000 lb., applied with fertilizer, produced a significant reduction in rot together with a decrease in bulb weight that was serious only when $Al_2(SO_4)_3$ exceeded 400 lb. In North Carolina, with soil initially at pH 5.3, significant differences in rot and bulb weight were not obtained. In some soils, the narcissus tolerates 100 lb. of HgO per acre without reduction of bulb weight.

(Cooperative investigations of U. S. Bureau of Plant Industry and Cornell University Agricultural Experiment Station.)

Nectria Canker in Relation to Growth and Mortality in Basswood (Tilia americana). D. S. WELCH. Growth studies were initiated in 1927 and 1928 on 10 half-acre plots in a stand consisting of 15 per cent to 20 per cent basswood. Final measurements were made

10 years later, when all trees were examined for Nectria canker. Those with lesions at or near the point of diameter measurement were discarded, leaving a total of 431 trees, 151 of which bore one or more trunk cankers of various ages. The average growth for all trees for the 10-year period was 0.86 in. Comparison with the average figure for basswood in the Lake States as given by Frothingham, 1.5 in. for a 10-year period, indicated that the trees were growing under unfavorable conditions. The average growth for the cankered trees was 1.03 in. against 0.77 for the trees not cankered. The results suggest that the more rapidly growing trees in a poorly growing stand are more likely to be cankered. During the same period, the dying of basswood in 2 of these plots bore no relation to Nectria infection but was directly correlated with amount of shading.

Differences in Cultural Characters and Pathogenicity of Strains of Tomato-wilt Fusarium. FREDERICK L. WELLMAN and DOROTHY J. BLAISDELL. A study was made of single-spore strains of 30 isolations from Fusarium-wilted tomatoes from widely separate regions of the United States. Some strains had been *in vitro* 15 to 30 years, others less than 3 months. In culture certain differential characteristics occurred fairly consistently, independent of origin. Differences were in mat growth and color, ranging from tough, slimy, appressed, and purplish to easily cut, woolly or cottony and white. Other differences existed. Seedlings of highly resistant tomato progenies (Red Currant), commercially resistant (Marglobe), and susceptible (Bonny Best), varieties were tested for response to *Fusarium* strains. Results were obtained through partly controlled greenhouse technique, evaluating individual plants numerically by dissection methods. All *Fusarium* strains proved pathogenic; cultures maintained on agar many years were as pathogenic as those of similar type recently isolated from wilted tomatoes; appressed strains were distinctly less pathogenic than raised; using appressed strains, commercially resistant tomatoes were infected, but they resisted rapid systemic invasion that caused relatively severe disease in susceptible plants; Red Currant tomatoes were resistant to all strains, but were slightly more infected by raised types. Single spore progenies of some raised forms were of both types. Pathogenically, they reacted as the appressed and raised forms.

Results of Barberry Eradication in Pennsylvania. L. K. WRIGHT and R. S. KIRBY. For the past several years a study has been conducted on the yield of oats in areas infested with barberry bushes before and after their eradication in Pennsylvania. In 89 fields growing near barberries the average yield before eradication was 20.30 bus. per acre. The average yield in the same or adjacent fields following eradication was 43.13 bus. per acre. Due to lack of certain grasses in Pennsylvania, such as *Hordeum jubatum,* and the presence of other grasses, such as *Dactylis glomerata* and *Agropyron repens,* the oats and rye strains of stem rust are more common and destructive than wheat strains. Stem rust has been destructive to wheat in certain grain-growing districts where barberry bushes were adjacent to wheat fields showing telial infection. In 1938 the wheat strain of stem rust was more widespread and destructive than for many years.

The Fungicidal Value of Cottonseed Oil and some other Spray Supplements. C. E. YARWOOD. The vegetable and animal oils, cottonseed oil, palm oil, coconut oil, castor oil, pine oil, corn oil, and sardine oil were more effective as supplements in increasing the protective action of Bordeaux mixture and the proprietary coppers, Basicop, Cuprocide, Coposil, copper phosphate, and copper zeolite for onion downy mildew in greenhouse tests, than the mineral oils, Ortho K light, Volc, and Ortho tank mix, or the spray supplements, Penetrol, Grasselli spreader or Lethane spreader. In 1938 field tests 0.5 per cent Bordeaux + 0.5 per cent cottonseed oil gave equal or better control of onion downy mildew than did other sprays tested, including rosin-lime sulphur. Cottonseed oil was more effective than mineral oil in reducing Bordeaux injury on beans and hops. In addition to their supplemental value to other sprays, several spray supplements, including cottonseed oil, sardine oil, Grasselli spreader, Areskop, and Alphasol, when used alone, show considerable protective value against certain powdery mildews, and slight protective value against certain rusts and downy mildews.

Mycorrhizae of Red Pine (Pinus resinosa) in Relation to their Environment and the Well-being of the Trees. HARLAN H. YORK. For a number of years, the writer has been noting that the mycorrhizae of red-pine trees in forest plantings on apparently unfavorable sites were abnormal or imperfectly formed. The plantings have been established 5 to 25 years. With few exceptions, only pseudomycorrhizae develop even though mycorrhizal-forming fungi are known to be present. Humification is poor in the older plantings where the trees eventually become attacked by *Polyporus schweinitzii* and other parasites; some of the latter infect [illegible] mycorrhizal and other fine rootlets. In such plantings, environmental conditions have arisen that appear to be antagonistic to the development of mycorrhizae.

Chemical Soil Treatment to Control Fusarium lycopersici, Heterodera marioni, and Weeds. P. A. YOUNG. In the last 3 years, chemicals were tested extensively to control parasites and weeds in sandy land, where Fusarium wilt kills most tomato plants, and root-knot nematodes damage most watermelon plants. Chloropicrin and carbon bisulphide were injected into the soil plats with a tube-peg-board and a Carbona Prod. Treated soil was covered during 3 days with glue-coated paper. Soil was tested by growing watermelons, Stone tomatoes, and Whippoorwill cowpeas in it during 2 to 3 months. At 250 to 450 lb. per acre, chloropicrin usually controlled all or most of the *Fusarium lycopersici, Heterodera marioni,* and weeds (especially *Sorghum halapense, Digitaria sanguinalis,* and *Amaranthus* spp.) At 1000 to 3000 lb. per acre, carbon bisulphide usually controlled nearly all of the *Heterodera marioni,* but did not control *Fusarium lycopersici* nor weeds. Carbon bisulphide at 500 and 800 lb. per acre and chloropicrin at 100 and 150 lb. per acre did not kill soil parasites. *Heterodera marioni* was not controlled by lye at 1470 lb. per acre, by sodium cyanide at 1200 lb. per acre, nor by cyanamide at 2000 lb. per acre.

Resistance of Tomato Varieties to Fusarium lycopersici. P. A. YOUNG. The following numbers give the economic wilt resistance of tomato varieties calculated as weighted percentages: Baltimore 58 to 74, Blair Forcing 84, Break O'Day 72, Brimmer 68, Browns Special 38, Buckeye State 84, Century 40 to 85, Early Baltimore 71, Early Detroit 33, Everbearing Scarlet Globe 61, Extra Early Prolific 58, Globelle 57 to 72, Golden Queen 53, Grothens Red Globe 77, Gulf State Market 48 to 63, Illinois Baltimore 91, Illinois Pride 76, Indiana Baltimore 64, John Baer 33, Kanora 65, Long Calyx Forcing 75, Louisiana Dixie 73, Louisiana Gulf State 54, Louisiana Pink 90, Louisiana Red 89, Marglobe 73 to 91, Marhio 85, Marvel 80, Marvana 70, Michigan State 78, Newport 78, Pink Glory 62, Prairiana 73, Pritchard 74, Red Cherry 51, Red Rock 11, Riverside 83, Rutgers 82, Stone 26, Sureset Forcing 80, Summerset 31 to 57, Wenholz Australian crosses of Earliana with *Lycopersicon pimpinellifolium* 10 to 77, Yellow ponderosa 72, and White-flower-Marglobe 78. This selection of X-ray treated Marglobe segregated with the white-flower character recessive in 1–3 Mendelian ratio to the yellow-flower character.

Two New Viruses Affecting Pea. W. J. ZAUMEYER. In 1938, Whipple and Walker reported two strains of the cucumber virus on pea and bean. In 1937, 2 additional viruses of the cucumber type had been collected from peas growing in northeastern Colorado. One produces a killing of the terminal growth of peas, but no leaf mottling, and is designated as the dieback virus. The other causes a characteristic streaking of the stems and a mottling of the leaves, and is called the stem-streak virus. The dieback virus kills the inoculated leaves and, later, the terminal growth. The stems become only slightly streaked. Infected plants are about one fourth natural size. The stem-streak virus produces no killing, but the leaves are mottled, as with the several pea mosaics. This virus causes less plant stunting than does the dieback virus. Of 12 pea varieties inoculated with the dieback virus, all were extremely susceptible, while 5 were resistant to the stem-streak virus. Both viruses infected tobacco, cucumber, Sieva Lima bean, petunia, Windsor broad bean, but not the common garden bean. In these hosts, the dieback virus produces more severe symptoms than does the stem-streak virus. The latter is unlike the pea-streak virus described earlier by the writer.

Varietal Reaction of Peas to Septoria pisi. W. J. ZAUMEYER. In 1936 a mild epidemic of Septoria blight occurred in an experimental planting of peas in a mountainous region of northeastern Colorado. It was believed that the primary infection derived in this case from seed of foreign origin. Widespread dissemination was noted in sections of Montana in 1938. In the past 2 years, artificial inoculations were made by using the previous year's infected pea straw as the source of inoculum. The infected straw was immersed in water and the liquid used to spray young pea seedlings grown under field conditions. Widespread infection and dissemination occurred in the plantings. Approximately 120 to 150 strains and varieties of peas were tested in 1937 and 1938. Only a few varieties appeared to be tolerant. Blue Prussian, a foreign importation, showed the most resistance. Strains of Perfection variety were the least susceptible of any of the canning varieties that exhibited moderate infection. None of the market types tested exhibited any particular tolerance. Comparatively high humidities are essential for infection. Peas planted in soil where Septoria-infected refuse was plowed under became infected early in the season. Whether the infection was induced by pycnospores from the previous season's pea straw or from a perfect stage of the fungus, not yet found, is unknown.

A CYTOLOGICAL STUDY OF RESISTANCE OF VIKING CURRANT TO INFECTION BY CRONARTIUM RIBICOLA[1]

OLOF CAMPBELL ANDERSON

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INTRODUCTION

Many intensive investigations have been made on the life history and parasitism of *Cronartium ribicola* Fischer, the fungus that causes blister rust of 5-needle pines. Research, however, with respect to its alternate hosts, *Ribes* L. (currants and gooseberries), has been confined largely to testing these for susceptibility or resistance to rust infection. Cytological investigations of infected *Ribes* are not numerous and have been restricted to studies of susceptible species. Knowledge of the host-parasite relationships of resistant *Ribes* has been almost lacking in the literature.

Among the large number of *Ribes* spp. investigated both in Europe and nerica, only a very small number has been found to be highly resistant to or immune from blister rust. The Viking[2] (syn. Rød Hollandsk Druerips) red currant from Norway has been extensively tested by Hahn (12, 14, 15), both in the greenhouse and in the field, and found to be extremely resistant to rust infection. Evidence of infection in this variety is restricted to the appearance of necrotic flecks in young immature leaves; there is no record of the production of urediospores or teliospores on the infected leaves (12). The Viking currant, therefore, was selected for this cytological study in order to determine the manner of infection, the development of the parasite, and the reaction of the host tissues to the invading fungus.

HISTORY

De Bary (4) was the first to demonstrate that germ tubes from spores of rust fungi, including those of a species of *Cronartium*, enter leaves by way of the stomata. He also observed that, although germ tubes from spores of many species of fungi enter stomata of certain green plants, their further development ceases in some immediately after entrance. Since the work of de Bary, other investigators of the Uredinales have verified his observations

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[1] Revised and condensed from a thesis submitted to the Faculty of the New York State College of Forestry at Syracuse University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

The writer takes pleasure in the acknowledgment of his indebtedness to Dr. Ray R. Hirt for suggesting the problem and for his advice and criticism, and to Dr. Glenn Gardner Hahn, who has taken an active interest in the investigation and placed Viking material at the disposal of the writer.

[2] The origin of Viking is unknown (14). The variety closely resembles the rust-resistant variety Prince Albert, which is regarded as a form of the hybrid species *Ribes pallidum* Otto and Dietrich (*R. petraeum* Wulf. × *R. rubrum* L.). In shape the leaves of Viking are very characteristic and differentiate the Norwegian variety from susceptible garden currants. The Viking shows *petraeum* characteristics in its stout, erect stems and in its irregularly shaped, longer-than-broad, 3-lobe leaves (14, Pl. 1). Resistance to rust in this hybrid probably is inherited from the *rubrum* parent (16).

(9, 10, 19, 26). Tubeuf (27, p. 139) described the method by which germ tubes from spores of *C. ribicola* enter the stomata of *Ribes* leaves.

Clinton (6) reported artificial inoculation of susceptible *Ribes* leaves in Petri dishes. He traced germ tubes from aeciospores and from urediospores into the mesophyll by way of the stomata and showed that infection takes place in this manner rather than by direct penetration of the epidermal cells.[3] Colley (8) also found evidence of stomatal penetration in *Ribes* leaves, and raised the question as to whether the germ tubes enter the leaves only by way of the stomata. Spaulding (25, p. 51) stated, "York found germ tubes of aeciospores entering the stomata of leaves of *Ribes cynosbati, R. glandulosum,* and *R. nigrum.*"

Spaulding (25) and Spaulding and Gravatt (24) reported the comparative susceptibility of a large number of *Ribes* species, the leaves of which had been inoculated artificially with spores of the blister rust fungus. They found *R. alpinum* L. entirely immune, although it is reported to have the disease in Europe. *R. leptanthum* A. Gray and *R. innominatum* Jancz. were highly resistant; *R. petraeum,* which probably is one of the parents of the Viking, was classed as of medium susceptibility. Spaulding (25, p. 24) recorded the following varieties of cultivated red currants to be resistant but not entirely immune: Eyath Nova, Franco-German, Holland, London, Rivers, and Simcoe King. He found varieties of cultivated gooseberry, *R. reclinatum* L., somewhat resistant. The resistance of these *Ribes* spp. was indicated by the occurrence of fewer and smaller uredia and by telia with a lowered viability. At times small flecks of dead or dying tissue appeared early in the infected leaves, but few, if any, uredia or telia developed.

Hahn (13, p. 114) also tested a large number of cultivated currants and found in a limited number of tests 9 varieties that showed a high degree of rust resistance, and 3 immune varieties: Franco-German, Holland, and Victoria. The Franco-German and Holland varieties he investigated were from sources other than those tested by Spaulding under these names.

The comparative susceptibility of a large number of *Ribes* spp. and varieties was studied also by Tubeuf (28). Among those that he found immune or near-immune from blister rust, was the red garden variety Rote Holländische, regarded by him as a form of the hybrid species *Ribes houghtonianum* Jancz., originated by crossing *R. rubrum* × *R. sativum* (Reichenb.) Syme, or the *petraeum* hybrid species *R. pallidum* Otto and Dietrich. He tested the Rote Holländische intensively over a period of 5 years (1928–1932), and proved it to be immune. Among his recommendations for the control of blister rust in Germany, Tubeuf stated that the rust-immune Rote Holländische was the only currant that should be widely planted in that country as a substitute for the black currant (*R. nigrum* L.) and other susceptible garden varieties (29, 30).

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[3] Through the courtesy of the late Dr. G. P. Clinton and Dr. F. A. McCormick, the writer examined their slides showing the entrance of germ tubes into the stomata of susceptible *Ribes* leaves.

About the same time that Tubeuf first reported the blister-rust-immune variety in Germany, Hahn (12) made a preliminary report on the resistance of the Viking variety known to Norwegian horticulturists as the Red Dutch currant (Rød Hollandsk Druerips). He found that its leaves became infected before they were fully mature and hardened. He described the chlorotic spots that developed and the small watery-appearing pustules that occurred beneath these spots in young leaves that had been inoculated with aeciospores. Hahn concluded that the fungus was able to enter the leaves of the Viking but was unable to propagate itself. Under greenhouse conditions he artificially inoculated Viking leaves with *Cronartium ribicola,* collected in the United States (14). In no case did fertile or abortive urediospores or teliospores develop in the leaves of Viking during these experiments, which extended over a period of 4 years. On the other hand, in the leaves of the susceptible *Ribes* "check plants," normal fruiting bodies of the blister rust fungus did develop.

Further confirmation of the resistance of Viking to *Cronartium ribicola* was obtained by Hahn (15) in field tests with the introduced variety, reported to have been immune from blister rust for many years in Norway. Over a period of 3 years (1932–1934), Viking remained resistant when subjected to rigorous testing under natural conditions. The field results fully substantiated the previous laboratory and greenhouse experiments. Hahn's belief that *C. ribicola* was able to penetrate the Viking leaf, but unable to produce spores, is now confirmed by the cytological evidence described in this paper.

MATERIALS AND METHODS

Three *Ribes* species, which varied in degree of susceptibility to *Cronartium ribicola,* were selected for comparison with the Norwegian currant: The highly susceptible *R. nigrum* L., the moderately susceptible smooth wild gooseberry, *Grossularia hirtella* (Michx.) Spach, and the *R. sativum* hybrid variety, American Red Dutch.[4] The black currant was propagated from cuttings obtained near Syracuse, New York; the wild gooseberry bushes were dug locally while dormant; and the American Red Dutch currant bushes were propagated from cuttings obtained at Geneva, New York.

The Viking material made available to the writer for this study consisted of plants propagated from cuttings from Ås, Norway, by the U. S. Department of Agriculture. They had been used since 1932 for testing blister-rust resistance at Chestertown, New York (15, Fig. 1, A). The plants of the susceptible species and of Viking were transferred in dormant condition to sites near Glen Haven and Dewitt, New York, where some were planted in pots or large pails and others, in the open ground.

Aeciospores for inoculation were obtained from cankered northern white pine, ***Pinus strobus*** L., growing in the Charles Lathrop Pack Demonstration Forest at Warrensburg, New York. Urediospores were obtained from heavily infected leaves of ***Ribes nigrum*** growing at Syracuse, New York, and Oshawa, Ontario, Canada, and from leaves of ***R. glandulosum*** Grauer, collected at Seventh Lake, New York. The method used to inoculate the ***Ribes*** followed closely that described by Hahn (11). For the purpose of localizing inoculated leaf areas, rectangles, approximately 1 cm. square, were outlined with water-proof India ink on the upper surface of selected leaves. Aeciospores were placed by means of a soft camel-hair brush on the under surface directly beneath the marked squares. On each vigorous and rapidly growing bush 12 to 15 representative leaves were inoculated, including young, fully expanded leaves, and older hardened leaves. Following inoculation the entire plant was sprayed with sterile tap water. This procedure was modified slightly when transferring urediospores to the marked leaves; small portions of currant leaves with newly erupted uredia were gently rubbed on the under surface, which had previously been atomized with sterile tap water. Each inoculated bush was then inclosed for 48 hours in a muslin damp-chamber within which a high relative humidity was maintained.

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[4] The American Red Dutch is not to be confused with the Red Dutch varieties of Europe from which it is distinct botanically. For convenience in this paper the American Red Dutch variety is called *Ribes sativum,* although the correct usage would be *R. sativum* hybrid variety, American Red Dutch. The writer is indebted to Mr. George L. Slate of the New York Agricultural Experiment Station at Geneva, New York, for cuttings of this variety.

Leaf material was removed for microscopic examination at definite intervals following inoculation. In 1935, material upon which aeciospores had been placed was collected every 24 hours during the first week, and every few days thereafter during the month of June. Material that had been inoculated with urediospores was collected on the same schedule through July and August. In 1936, portions of leaves were collected 24, 36, 48, 60, 72, 120, 240, and 360 hours following inoculation with aeciospores in June and with urediospores in July.

The development of the rust was studied from cleared sections of leaves, as well as from stained permanent mounts. The leaf sections were cleared by a modification of the method described by Peace (20). The differentiation of the germ tubes and appressoria on the leaf surface was accomplished by adding a drop of 1 per cent acid fuchsin to the mount.

The material for permanent mounts was fixed either in formalin-acetic acid-alcohol or in Fleming's solution. The formalin-acetic acid-alcohol mixture was compounded by volume of 5 cc. formalin C.P., 7 cc. glacial acetic acid, and 88 cc. of 50 per cent alcohol. Complete fixation usually occurred in 24 to 48 hours. Zirkle's (33) method of dehydrating and clearing was followed, and the samples were embedded in paraffin. Sections 6 to 8 μ thick were cut and mounted serially on numbered slides. Safranine-O in 50 per cent alcohol proved to be a satisfactory differential stain when followed by a short counter stain of 1 per cent fast green in alcohol.

ANATOMY OF THE VIKING LEAF

The normal leaves of Viking do not differ in gross anatomy from those of ***Ribes nigrum, R. sativum,*** and ***Grossularia hirtella.*** The upper epidermis lacks stomata and is composed of flat irregular-shape cells. The mesophyll consists of typical palisade and spongy parenchyma tissues. The palisade cells are cylindrical or truncated cone-shape and loosely arranged in single or double rows. The spongy parenchyma cells are loosely arranged, and in transverse section may appear regularly or irregularly round or ovoid in outline; in surface view they are irregularly lobed. Cells filled with a granular substance are interspersed among the palisade and spongy parenchyma cells, at times forming a more or less definite layer in the latter tissue. Remnants of chloroplasts and nuclei can be faintly discerned in these granular cells. The lower epidermal layer has an average of 213 stomata per sq. mm. The stomatal pores average 4.0×8.3 μ, when open, and are not oriented in any particular direction. The size of open stomatal pores and the number of stomata per unit of leaf area in the Viking are not significantly different from those of the fully susceptible species. Leaf hairs are present but not abundant on the ventral surface. Stalked glandular hairs occur sparingly on the upper epidermis.

THE DEVELOPMENT OF INFECTION AREAS

In the fully susceptible *Ribes* studied, the first macroscopic evidence of infection[5] is the development of chlorotic spots within approximately 6 to 8 days after inoculation. Two to 4 days later, urediospores are produced on the underside of the leaves, beneath the chlorotic areas.

The symptoms of infection in the leaves of the resistant Viking have been described by Hahn (12, p. 144 and 145). The writer's experience has shown that, in general, these evidences of infection were rather constant. Infection usually could be detected about 4 days after inoculation, especially when the leaves were viewed by transmitted light. On the upper surface of the leaves infection first appeared in the form of chlorotic spots, beneath which, on the under surface small watery pustular swellings developed. Approximately 2 to 6 days later necrosis developed near the center of each pustular swelling. Gradually the chlorotic areas enlarged, resulting in islands of necrotic tissue surrounded by broad chlorotic bands (Fig. 1). By the end of the summer the necrotic areas increased to as much as 0.5 cm. in diameter, and included both epidermal and mesophyll tissue.

External evidences of infection in the Viking were restricted to young, completely expanded, tender leaves; fully mature, hardened leaves, with a single exception, failed to show any evidence of infection. A series of inoculations with urediospores were made on 3 bushes of Viking currant, the leaves of which had the appearance of being mature, *i.e.*, they were apparently hardened, of leathery texture, dark green in color, and at least 4 months old. On one of the bushes, which grew in a shaded location, 15 leaves showed typical necrotic spots. The 2 remaining bushes, which were growing in the open, failed to develop either chlorotic areas or necrotic flecks, even though microscopic examination of the leaves disclosed that germ tubes of the spores had actually penetrated the leaves by way of the stomata. Evidently the leaves of the shaded bush, although mature, were not hardened to the extent of those growing on plants in the open.

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[5] Infection, as the term is used in this study, refers to all observable structural abnormalities of the host tissue as a direct or indirect result of the presence of the pathogen.

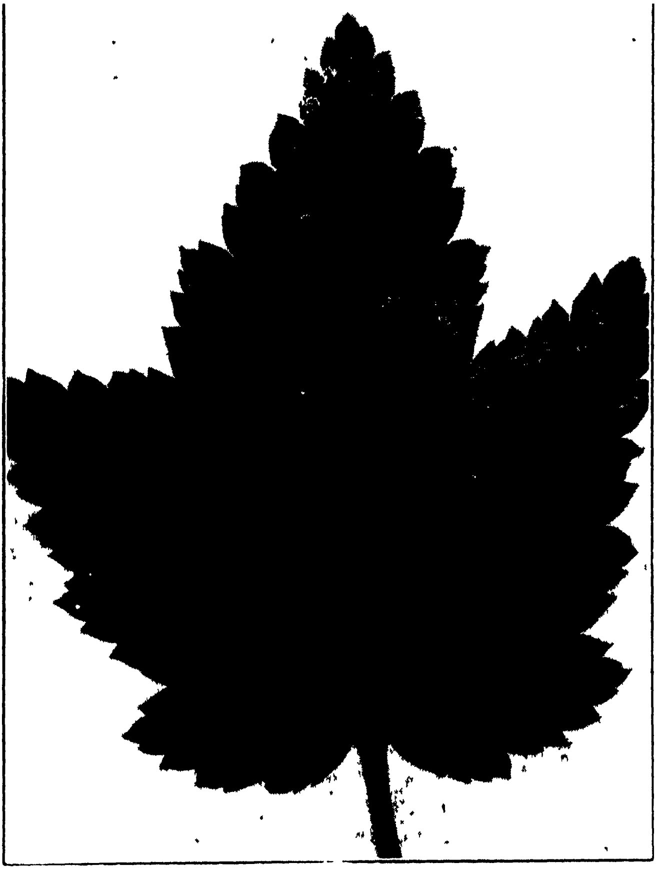

FIG. 1. Young leaf of Viking currant (underside), showing the necrotic flecks and chlorotic tissue consequent upon infection by *Cronartium ribicola.* Neither uredia nor telia developed in these areas. Photograph by J. L. Lowe. × 2.

CYTOLOGICAL OBSERVATIONS ON SUSCEPTIBLE RIBES

a. Rust Incubation and Penetration

The germination of aeciospores and urediospores, and the penetration of *Ribes* leaves by the young germ tubes were observed in portions of cleared leaves and in permanent mounts of transverse sections of leaves from each of the 3 *Ribes* species. The invasion by germ tubes from both aeciospores and urediospores was accomplished similarly. Twenty-four hours after leaves were inoculated the spores had germinated and the germ tubes elongated as much as 400 μ. The germ tubes grew at random over the surface of the leaves, at times passing near to and over stomatal openings without penetrating them; at other times they formed appressoria directly over the stomata. These were usually ovoid and varied slightly in size. A septum developed in the germ tube between the spore and the appressorium but nearer the latter. From beneath each appressorium a hypha grew into the stomatal opening, and at times could be observed growing across the stomatal chamber into the mesophyll. Among the many examples of penetration through stomata, observed by the writer, only once was a substomatal swelling, or vesicle seen. This vesicle developed in a germ tube from an aeciospore that had germinated directly over a stoma; the invading hypha had enlarged into a rounded vesicle immediately inside the stomatal chamber. The lack of substomatal swellings or vesicles in hyphae is not in agreement with the observations of Clinton and McCormick (7), although morphological variations in substomatal vesicles of invading hyphae have been reported in other rust studies (1, 9, 22). Thus it is not surprising that they should occur in hyphae of *Cronartium ribicola.*

b. Infection

After the invading hyphae had penetrated through the stomata, they branched profusely and grew into the intercellular spaces of the mesophyll. The mycelia developed in abundance and soon filled the air spaces, but no distortion of the leaf tissue occurred until later. Haustoria were not seen until the mycelium was approximately 240 hours old. The uredia began to develop in the substomatal chambers within this period. These chambers became filled with mats of closely intertwined hyphae. From the sclerotium-like formations, hyphae projected outward and formed large closely compacted and vertically arranged cells. These large cells developed into urediospores and a peridium. The epidermis over each sorus was pushed outward by the development of the uredia. Within 360 hours the peridium and the epidermis ruptured over each sorus and released the urediospores. These observations agree essentially with those of Colley (8, pp. 634 and 635).

As this extensive development of the parasite proceeded, very little deleterious effect upon the host tissues was observed. Distintegration of the chloroplasts was the first visible result of the presence of the hyphae among the spongy parenchyma cells. The nuclei of some of these cells enlarged, and frequently appeared along the sides of the cells nearest the hyphae. Following the disintegration of the chloroplasts and the enlargement of the nuclei, the entire contents of some of the cells broke down, while in others the enlarged, distorted nuclei persisted. Occasionally, in the leaves of *Ribes nigrum,* a slight disintegration of chloroplasts in cells near invading hyphae was observed 36 hours after inoculation. By this time the urediospores had formed and extensive areas of empty cells were observed surrounding the sori. In the leaves of *R. sativum* and *Grossularia hirtella* the disintegration of chloroplasts in the spongy parenchyma cells near invading hyphae was not observed until uredia had formed, about 240 hours after inoculation. A few intracellular hyphae were then present. In *G. hirtella* this breakdown of the chloroplasts was confined to the spongy parenchyma in the immediate vicinity of the uredia; the nearby palisade cells were unaffected. In *R. sativum* evidence of infection was observed in wider areas of the mesophyll about the uredia, but less extensive than in *R. nigrum.* By comparing the effect of hyphae on the tissues of the 3 hosts, it is seen that the reactions of the host cells are similar in character, but most cells were affected in *R. nigrum,* fewer in *R. sativum,* and fewest in *G. hirtella.* In none of these species did the mesophyll cells become enlarged.

CYTOLOGICAL OBSERVATIONS ON RESISTANT VIKING

a. Rust Incubation and Penetration

The germination of aeciospores and urediospores, and the method of penetration of stomata by the young germ tubes, were the same on the Viking (Fig. 2, A and B) as on the susceptible species. Occasionally, an invading hypha became broader as it passed between the guard cells, so that the long diameter of the hypha was parallel to the long axis of the pore (Fig. 2, B). This broadened hypha was not interpreted by the writer as a substomatal vesicle. From the stomatal pore the hyphae usually grew directly across the stomatal chamber, and continued intercellularly among the spongy parenchyma and palisade tissues (Fig. 2, C). In the Viking, branching of the intercellular hyphae occurred among the palisade cells, or bordering directly upon them, whereas in other species, branching first occurred in the stomatal chamber or among the spongy parenchyma cells, and later among the palisade tissues. The contents of the invading hyphae soon became granular and vacuoles developed. The nuclei, in comparison with the diameter of the hyphae, were smaller than those illustrated by Colley (8, pl. 59. Fig. C.). Large nuclei were present in young portions of the hyphae, such as wedge-shape hyphal tips (Fig. 2, C.). Delayed germination and penetration occurred as many as 7 days after inoculation; thus the early stages of the penetration phenomena were observed for some time after inoculation.

b. Infection

The invading hyphae in leaves of the Viking branched only sparingly in the mesophyll; they were never sufficiently extensive or abundant to fill the intercellular spaces, as in the leaves of the susceptible species of *Ribes* studied. About 48 to 72 hours after inoculation the hyphae gave evidence of degeneration in Viking, whereas in the fully susceptible *Ribes* species they were in a thriving condition. The older portions of hyphae near the stomata were often vacuolate, shriveled, and sometimes collapsed. The younger parts of hyphae were turgid and retained their contents for as many as 72 hours, but their nuclei became small and in many cases the cytoplasm appeared granular. Mycelium never developed extensively, and, within 96 hours, it had completely broken down and disappeared.

The cells of the invaded mesophyll of the Norwegian currant reacted very definitely before the disappearance of the hyphae. The first manifestation of

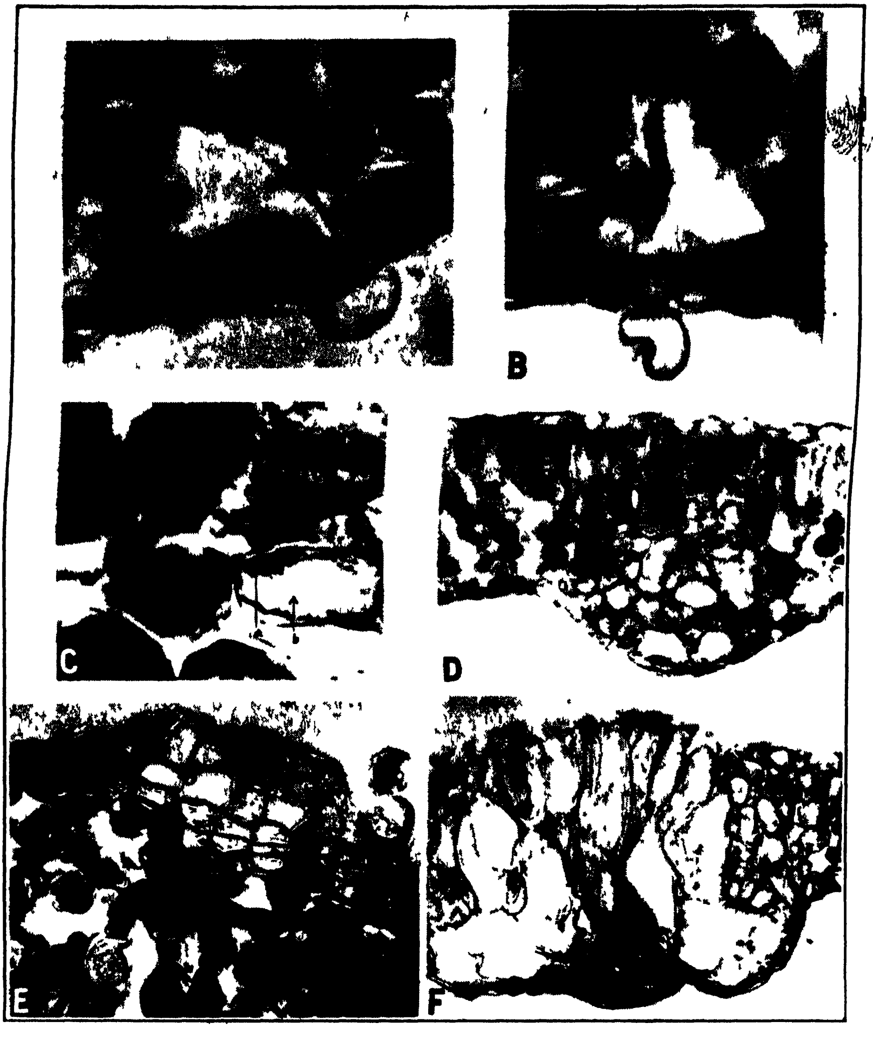

FIG. 2. Cross sections of Viking currant leaves after inoculation with spores of *Cronartium ribicola*. A. Appressorium and wedge shape hypha entering a stoma. × 835. B. Appressorium and a broadened hypha entering a stoma. × 700. C. Hyphal tip with a large nucleus (a) growing between palisade cells (b). × 835. D. Hypertrophy that developed in 5 days. The enlarged mesophyll cells lack chloroplasts, the air spaces are obliterated, and the epidermis is distended. × 200. E. Hypertrophy that developed after 6 days. A layer of meristematic tissue occurs near the enlarged mesophyll cells, which have enlarged nuclei and lack chloroplasts. × 310. F. Hypertrophy that developed after 10 days. A mass of collapsed cell walls form a portion of a core that extends among the enlarged mesophyll cells. × 310.

infection was the disintegration of the chloroplasts in the spongy parenchyma cells near the invading hyphae, which occurred as early as 48 hours after inoculation. The nuclei of the mesophyll cells adjacent to invading hyphae enlarged during the time that the chloroplasts were breaking down. Frequently, the enlarged nuclei were observed along the cell walls nearest the invading hyphae.

Within 72 to 96 hours after inoculation, and after the hyphae had disappeared, groups of enlarged cells were frequent in the spongy parenchyma of young leaves. Enlargement of palisade and spongy parenchyma cells sometimes occurred simultaneously, or enlargement first appeared in the spongy parenchyma cells and later in the palisade tissue. The intercellular spaces disappeared as cell enlargement continued; eventually, the lower epidermis was pushed outward. The area of hypertrophied cells, covered by this distended epidermis, constituted the watery-appearing pustular swelling previously mentioned (Fig. 2, D). Empty and collapsed appressoria were frequently observed outside stomatal pores in the distended epidermis covering the enlarged cells. They were the only evidencs of the pathogen observable in the immediate vicinity of the pustular swellings. In the zone surrounding the enlarged cells, the disintegration of the chloroplasts and the enlargement of the nuclei occurred.

Among the groups of enlarged cells a layer of meristematic tissue sometimes formed in the region of the spongy parenchyma when the infection was approximately 120 hours old. This later was composed of homologous, brick-shape cells that divided parallel to their long axes, and formed a roughly aligned stratum (Fig. 2, E.). The meristematic cells frequently were filled with a dense granular substance in which the nuclei could be faintly discerned.

Empty cells, frequently with collapsed walls, appeared among the groups of enlarged cells 72 to 240 hours after inoculation. These necrotic cells appeared first in the region near the lower epidermis; later, they occurred throughout the mesophyll tissue in the center of the hypertrophied area. The lower epidermis, which was pushed outward by the enlargement of the underlying mesophyll cells, sometimes ruptured above the necrotic regions. The collapsed epidermal cell walls formed the surface of the previously mentioned necrotic flecks.

Some groups of enlarged cells never developed a meristematic layer. In these groups both palisade and spongy parenchyma cells enlarged simultaneously at right angles to the surface of the leaves. This enlargement produced groups of long narrow cells that extended from the lower to the upper epidermis with little or no differentiation. The elongated cells were without chloroplasts and their nuclei had either enlarged, become appressed to the walls, were distorted, or had vanished. In the center of these hypertrophied areas, some of the enlarged cells died and formed a core of dead cells through the mesophyll from the lower to the upper epidermis. The epidermal layer was pushed outward and frequently was ruptured over the core to form necrotic flecks (Fig. 2, F.).

The necrotic area in the center of each of the lesions continued to increase in diameter for approximately 360 hours after inoculation. After the collapse of many of the dead cells in the underlying mesophyll, the broken-down walls of the epidermis became depressed. The enlarged mesophyll cells surrounding the dead areas that did not collapse were filled with a dense granular substance in which tylosis-like structures occurred. The lesions continued to widen for as many as 28 to 31 days, and the necrotic areas became separated from the surrounding unaffected tissues by rings of hypertrophied cells.

DISCUSSION

Intensive research concerning the nature of the resistance of green plants to infection by rust-producing fungi has been restricted largely to cereals and fruits. From these studies it appears that resistance may be of 2 kinds; (a) physical, and (b) physiological. The leaves of the Viking appear to offer no physical resistance to invasion by *Cronartium ribicola,* at least no more than the leaves of *Ribes nigrum, R. sativum,* and *Grossularia hirtella,* within which the fungus readily becomes established. In each of the 4 species the fungus was found to penetrate the leaves in a similar manner only by way of the stomata. Stakman (26), Newton (18), and Allen (2) also found entrance phenomena to be essentially similar in resistant and susceptible varieties of wheat attacked by *Puccinia graminis* Pers. and *P. graminis tritici* Erikss.

In the Viking the dimensions of the open stomatal pores, and the average number of stomata per unit of leaf area, were not significantly different from those of any of the 3 fully susceptible species. In all species the open stomatal pores were sufficiently large to permit penetration of the germ tubes, and the total pore area per unit of leaf surface was about the same in each of the *Ribes* species. Thus the size and number of stomata is not apparently a factor in the variation in infection among the 4 species. Hursh (16), in his study of the resistance of wheat to *Puccinia graminis tritici,* and Ward (32), in his study of resistant brome plants, both concluded that stomatal numbers were not correlated with resistance.

The existence of a physiological type of resistance in leaves of the Viking has been indicated by the incompatibility in the host-parasite relationship. After an entirely normal entrance, the hyphae of this obligate parasite are unable to become established in the mesophyll. As in plants resistant to certain other rust fungi studied by Allen (2, 3), Gibson (10), Marryat (17), Sharvelle (24), and Ward (31), the invading hyphae in the Viking died soon after gaining access to the mesophyll. This early death of the hyphae was explained by these investigators on the basis of a toxin having been formed. They presumed that the breakdown of a parasitized cell protoplasm produced a substance toxic to the hyphae, and thus they were made dependent upon their own reserve food, which soon became exhausted. Although the leaf cells of Viking do not die until after hyphae are broken down, it may be that a deleterious substance, such as a toxin, may diffuse from the affected cells and cause the death of the hyphae. The proof of this awaits biochemical study.

Not only do the young hyphae from the spores of *Cronartium ribicola* die soon after entering the leaves of the Viking, but they also disappear from the area of invasion. This agrees with observations made by other investigators (1, p. 145, and 24, p. 88) of rust fungi; however, in the Viking the hyphae disappear much sooner following invasion of the host tissue than is usual for other rust hyphae in their resistant hosts. These workers advance no satisfactory interpretation of these phenomena, and the writer can propose no valid explanation for the disappearance of the dead hyphae.

The death of hyphae that invade resistant host plants may occur as a result of starvation, either because haustoria fail to develop, or because the hyphae are surrounded by dead cells from which food cannot be obtained. During the first few days after invasion, haustoria did not develop in the leaf tissues of the Viking nor in those of *Ribes nigrum*, *R. sativum*, and *Grossularia hirtella;* yet the fungus was able to persist only in the tissues of the 3 latter species. Rice (21) suggests that haustoria may not be necessary to certain rust fungi during the early stages of infection in order for them to obtain food from their hosts, because they may be able to take nourishment directly through cell walls. In the Viking this does not appear to be true, inasmuch as the hyphae die while surrounded by living host cells. Stakman (26), Newton (18), and Allen (1) conclude that the hyphae of wheat rust starve in resistant plants because the cells of the host immediately surrounding the invaded areas are killed and thus offer no living food for the obligate parasite. This is not the case in the Viking, since the death of the host cells occurs several days after the hyphae have disappeared. Since the hyphae are closely surrounded by living host cells in all 4 species of *Ribes*, and since the hyphae develop abundantly in *Ribes nigrum*, *R. sativum* and *Grossularia hirtella* but die in the leaves of the Viking, it appears that an antagonism or incompatibility, *i.e.*, physiological resistance, exists in the leaves of the Viking.

The tissues of the Viking currant react to the presence of the hyphae. Evidences of incompatibility, indicated by the disintegration of the chloroplasts and the enlargement of nuclei in mesophyll cells near invading hyphae, were consistently present within 48 hours. In other species of *Ribes* these indications of incompatibility were not generally observed until much later. Stakman (26) considered similar disintegration of chloroplasts in wheat plants as an indication of incompatibility and hypersensitivity. The nuclei, which were frequently observed resting along the walls nearest invading hyphae in leaves of the Viking, were much enlarged; this enlargement may have been due to increased metabolic activity. Allen (2) discovered that the enlarged nuclei in infected wheat occurred in the least impoverished cells, and she suggested that these enlarged nuclei indicated an increased metabolism. Caldwell and Stone (4) advanced another hypothesis to explain the enlarged nuclei that they found in the accessory cells of the stomata and other epidermal cells in infected wheat plants. They believed their enlargement and orientation nearest the invading hyphae indicated either a chemical stimulus or a traumatic response.

The necrotic areas in the Viking differ greatly from the dead areas in the leaves described by Colley (8, p. 651). He states, "In the leaves of *Ribes* spp. the mycelium sometimes causes the death of isolated infected spots; but in other cases the hyphae penetrate to all parts of the tissue without causing the death of cells, and without producing hypertrophy." Spaulding (24, p.

54) noted isolated areas in *Ribes* leaves that became necrotic where "the attack of *Cronartium ribicola* is intense." In the infected leaves of the Viking, on the other hand, necrosis, which occurs in the center of hypertrophied areas, develops subsequent to the disappearance of the pathogen. The necrotic cells never are invaded, and their death is related only indirectly to the former presence of the hyphae. Hyphae are absent and cell hypertrophy is always present.

On the basis of the results of this investigation, Viking apparently is not directly dangerous to 5-needle pines, since the fungus cannot become established in its leaves, which are an unsuitable infection court for the hyphae of *Cronartium ribicola*. It is, therefore, not a source for the spread of the rust, either to other *Ribes* or to 5-needle pines, because the hyphae cannot reproduce or survive in its leaves. Where the propagation of Viking is restricted to cuttings the variety may be distributed with safety as a substitute for susceptible cultivated *Ribes* now being grown. Inasmuch as the Viking is a desirable red currant from a horticultural standpoint, it should serve as a valuable stock for the breeding of other desirable blister-rust-resistant varieties.

SUMMARY

A cytological investigation of the leaves of the blister-rust-resistant Viking (syn. Rød Hollandsk Druerips) red currant from Norway inoculated with *Cronartium ribicola,* demonstrated that infection took place in young, fully expanded, unhardened leaves through the stomata. The sparse hyphae, however, that arose from the invading germ tubes died before the parasite was able to develop a mycelium capable of producing urediospores or teliospores. On the other hand, fully matured, hardened leaves, with one exception failed to show any evidence of infection, although germ tubes had actually penetrated the leaves through the stomata.

As a result of the penetration and brief presence of meager blister-rust hyphae in the tissues of immature Viking leaves, minute necrotic lesions or flecks developed subsequent to the death and disappearance of the rust mycelium.

The cytological changes in the protoplasm of the parasite and in the Viking tissues affected by blister-rust infection are described.

The resistance of leaves of Viking to infection by *Cronartium ribicola* would appear to be physiological rather than physical; for the noninfected leaves of the Norwegian currant did not differ in gross anatomy from those of 3 susceptible currants in whose leaves fertile uredia formed readily. The susceptibles used for this study were the black currant, *Ribes nigrum,* the smooth wild gooseberry, *Grossularia hirtella,* and the *R. sativum* hybrid variety, American Red Dutch. It was found that the blister-rust fungus entered through the stomata of these susceptible species and produced fruiting bodies after the parasite had developed abundantly among the host tissues.

The explanation of the apparent antagonism existing between the leaf tissues of Viking and the hyphae of *Cronartium ribicola,* probably is to be found in a biochemical study of the parasite and its resistant host.

Additional corroboration of the resistance of Viking to blister rust is given by this cytological study. The new data obtained serve to substantiate the results of extensive inoculation and field tests in which the Norwegian variety was demonstrated to be extremely resistant and prohibitive to the development of the fungus spores.

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A STUDY OF VIRUSES INFECTING EUROPEAN AND AMERICAN VARIETIES OF THE POTATO, SOLANUM TUBEROSUM

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INTRODUCTION

The potato, *Solanum tuberosum* L., has been reported as being affected by a score or more of fairly distinct viroses. These separations have been based largely on symptom expression that, as now generally known, is not always an entirely reliable criterion. The subject also has been greatly complicated by differences in varietal reactions to specific viruses, the varieties used for the symptom descriptions being especially different in Europe and America. This varietal behavior has not been altogether a handicap to differentiation, since, when satisfactorily used, it presents added criteria in the hands of the investigators. In recent years more emphasis has been placed on closer attention to the description of the specific effects of the virus itself, made doubly desirable in the potato diseases because of the frequent causal relationship of 2 or more viruses with a single disease expression. This, in turn, is attributable largely to the fact that, the potato is a symptomless carrier of one or more viruses.

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The need for more comparisons of the different viruses, described by investigators under widely different conditions, through an exchange of materials, has become increasingly evident. Some efforts already have been made by others with good results (15, 19, 21), but it is obvious that several intensive comparisons of this type must yet be made before a complete understanding of this important virus group may be expected.

The present comparative study was undertaken to determine the identity of the potato viruses found here and those known in Europe. These viruses have been studied under identical conditions on different American and European potato varieties, in many cases on plants that came from the same tuber unit, as well as on other Solanaceous host plants. In addition, some of the physical and chemical characteristics of these viruses have been determined.

These studies were initiated in the fall of 1933 at Corvallis, Oregon, and conducted in the greenhouse and under insect-proof cages in the field. A preliminary report of this work already has been published (8, 9). In the spring of 1936, the writer was transferred to Beltsville, Md., where the studies were continued in the United States Horticultural Station.

EARLY STUDIES

The early work on the classification of the different potato viroses found in this country was conducted by Schultz and Folsom (30), and that in Europe by Quanjer (23). In most of the research in the United States the variety Green Mountain was used. At that time it was not so fully realized that such a variation of symptoms from the same virus might develop on different varieties of the potato. It is now known that the symptoms caused by viruses are influenced by the host and environment; therefore, it is not possible to develop a general system of classification on the basis of symptoms alone. The term "mild mosaic" for example, is applicable to the symptoms produced by a known virus in a number of varieties of potato; but in at least 2 varieties, *i.e.*, Up-to-Date and British Queen, it produces a definite acronecrosis. The term "latent virus of healthy potatoes" originated when it was believed that this virus was latent in all varieties. We know now that this virus causes a definite mosaic-like mottling in such varieties as President and Arran Victory and a severe top necrosis in Epicure and Katahdin. Whereas, formerly, a system was developed only to classify the diseases caused by the different viruses, the need is now felt for a classification of the different viruses causing the diseases. Johnson (12) attempted to identify and differentiate potato viruses by the determination *in vitro* of certain physical and chemical characteristics, such as the thermal death point, the longevity *in vitro,* the effect of dilution, and the influence of certain chemicals. Quanjer (24), although agreeing that the determination of these properties offers great possibilities for acquiring knowledge of the nature of the viruses concerned, believes that such studies cannot be expected to give much information on their properties as pathogens.

Quanjer also believes that a potato virus should be identified, named, and classified according to the morbid effect it has on a variety that shows clearly definable internal symptoms. He considers President one of the best differential hosts. To avoid further trouble resulting from the use of descriptive names for an ever-increasing number of mosaics, crinkles, and streaks, he suggests the following as an international system of nomenclature for the potato diseases of the virus type.

Anecrotic mosaics: producing only mottling and more or less wrinkling of leaflets, without necrosis.

Acronecrosis (*top necrosis*) : necrosis radiating from only a rather small percentage of the internal phloem strands into the surrounding parenchyma.

Acropetal necrosis: necrosis chiefly in the collenchyma of the leaf veins, petioles, and stems extending gradually to other tissues; dropping of lower leaves.

Phloem necrosis: necrosis restricted to the phloem strands, *i.e.*, sieve tubes and companion cells, accumulation of carbohydrates in the leaves.

Phloem parenchyma necrosis (*pseudo net necrosis*) : necrotic spots in the storage parenchyma, next to the external and internal phloem of the tubers only.

Although realizing that these histopathological changes offer a promising field for research on the pathogenicity of viruses, Bawden (2) fails to understand the advantage gained in using the morbid anatomy of infected plants as criteria for classification rather than the external symptoms, which he considers more definite and much more easily recognized than the internal changes.

In 1931 Smith (31) designated as X the virus causing the disease known in Europe as simple mosaic, and, in America, as "latent mosaic of healthy potatoes." He designated as Y the virus causing acropetal necrosis, the common leaf-drop streak in President. Murphy and McKay (18) described virus A, which they found occurring alone in apparently healthy plants of the variety Irish Chieftain, and which, together with the virus X, causes the potato disease known as crinkle. Bawden (unpublished thesis, Smith's book) (32), described virus B, seemingly almost universal in the variety Up-to-date, which carried it without symptoms, but usually accompanied by virus X. Virus B, when transmitted by grafting produces a top necrosis in several varieties. Bawden (2) also described a virus C as occurring in the variety Di Vernon. Scions from infected Di Vernon plants when grafted to virus-free Arran Victory and President plants, produce top necrosis in the latter, while the former reacts with mosaic symptoms only. The non-production of top necrosis in Arran Victory differentiates this virus from virus B. Virus D also has been described by Bawden. This virus is closely related to virus X, and is apparently an aberrant strain of it. It causes top necrosis in different varieties of the potato, all of which carry either virus X or show only interveinal mottle when infected with it. Clinch, Loughnane, and Murphy (8) designate the virus causing tuber blotch as F, and that of aucuba mosaic as potato virus G. These authors, by taking a general view of their properties and reactions, classify the potato mosaic viruses into three broad groups, namely:

1. Viruses of the X type, which cause distinctive mosaic symptoms in many solanaceous hosts; produce crinkle when combined with virus A in varieties of potatoes tolerant of that disease and are non-transmissible by *Myzus persicae.*
2. Viruses of the F type (tuber blotch virus) which produce characteristic brown or purple fringed spots on *Solanum nodiflorum,* produce typically bright yellow spotting of the lower leaves of potatoes that is intensified by the presence of virus A, and cause hereditary parenchyma necrosis in the tubers of many varieties of potatoes.
3. Viruses of the Y type, which are readily transmissible by *Myzus persicae,* produce green veinbanding in tobacco, are noninoculable to *Datura stramonium,* and, typically, cause acropetal necrosis in certain varieties of the potato, although this feature varies in the case of virus A.

MATERIAL AND METHODS

In 1924 diseased Green Mountain tubers were furnished to the Oregon Experiment Station by E. S. Schultz of the United States Department of Agriculture, from the experimental stocks grown at the Field Station at Presque Isle, Maine. Each of these tubers was infected with one disease only, *i.e.,* mild mosaic, crinkle mosaic, rugose mosaic, and leafrolling mosaic. Since then, this material has been propagated on different varieties under muslin-covered cages in the field, and was the source of the American viroses investigated.

In 1933 the Y virus and Crinkle A in the variety President, Para crinkle on Arran Victory, virus C in Di Vernon, the virus B in Up-to-Date, and healthy tubers of President, Arran Victory, and Majestic were obtained from R. N. Salaman of the Potato Virus Institute of Cambridge, England. In 1936 the blotch virus in the variety President was received from P. A. Murphy of Glasnevin Agricultural College, Dublin, Ireland. At that time stipple streak in Zeeland Blue, pseudo net necrosis in President, and healthy tubers of British Queen were received from H. M. Quanjer of the Agricultural College, Wageningen, Netherlands.

The studies on the tuber blotch, pseudo net necrosis, Aucuba, and Calico viruses will be discussed in a later publication.

TECHNIQUE USED IN VIRUS TRANSMISSION

Various means of transfer of the several potato viruses were tried out. Certain methods were successful with some of the diseases but not so satisfactory with others.

Leaf Mutilation

In this method, as described by Schultz and Folsom (30), cheesecloth soaked with the juice from macerated foliage of diseased plants was rubbed on the leaf of a healthy plant, with enough pressure to break the epidermis or mash or mutilate the tissues to a degree. This method was successful in transmitting many of the viruses, but there was considerable variation in the amount of infection thus obtained from the different viruses. It was later found that the percentage of infection of these difficultly transmissible viruses could be increased by adding a small amount of carborundum dust to the inoculum (25). This acted as an abrasive and facilitated the entrance of the virus into the inoculated plant.

Stem Grafts

It was found that, with the exception of the veinbanding or Y group of viruses, the grafting method of transfer was far more successful than the leaf-mutilation method. Stem grafts of diseased scions were made to potted healthy plants that were kept in a moist chamber for about 10 days. They were then removed and kept in the greenhouse for further observations, according to the method recommended by Salaman and Le Pelley (26). When this procedure was followed, about 80 per cent of the grafts were successful.

SEROLOGICAL STUDIES

Beale (4), Chester (6), and others have carried on serological experiments demonstrating that the injection of rabbits with plant-virus extracts induces in such animals the production of antibodies. When the serum from the animal containing the antibodies is added to juice from a plant containing the same virus or that of a related strain, the soluble antibody of the serum and the soluble antigen of the virus juice combine to form an insoluble precipitate, which slowly settles out. Unrelated plant viruses fail to react. It is believed that this method provides a very useful means of determining the relationship between different potato viruses. Accordingly, a number of these viruses have been tested serologically through the courtesy of Dr. K. Starr Chester, formerly of the Rockefeller Institute for Medical Research, Princeton, New Jersey.

ACQUIRED IMMUNITY FROM PLANT VIRUSES

Several workers, namely McKinney (17), Thung (33), Salaman (28), Price (22), Holmes (11), and others, have found that there are different strains of viruses causing diseases that show a considerable degree of difference in severity. They found that plants infected with one strain are often thereby protected against infection by other strains of the same virus, including those of greater virulence. This technique has been used also in these studies to determine whether or not some of the different potato viroses are caused by strains of the same virus or by unrelated viruses.

STUDIES ON INDIVIDUAL VIRUS DISEASES

The discussion of the individual diseases brings together the results of observations and experiments conducted by the writer. The symptoms described are those observed under greenhouse conditions at temperatures varying from 65° to 75° F.

The number of plants used in the different experiments is not always stated, but, with few exceptions, a sufficiently large number of plants, generally not less than 20, was used in each test, and similar inoculations were often repeated.

Mild Mosaic

This disease was originally described by Schultz and Folsom (30). It is composed of at least one component in addition to virus X. The symptoms in Green Mountain potatoes are characterized by a mottling of the leaf in which yellowish or light-color areas alternate with similar areas of normal green. This usually is accompanied by a crinkling, but not a rolling, of the foliage. These mottled areas are variable in size and shape and located without regard to the different tissues such as the veins of the leaf. This is in contrast to the loss of color occasioned by other factors than mosaic, which may give rise to yellowish, somewhat circular, island-like areas of the leaf tissues between the larger veins. In mild mosaic the lighter colored areas are not bounded or stopped by leaf veins. Diseased plants droop and die prematurely. No symptoms are evident in affected tubers. This disease also has been studied in the American varieties, Burbank, Earliest of All, White Rose, and Bliss Triumph. Although varying slightly in intensity, depending upon the variety, the symptoms are not fundamentally different from those described in the Green Mountain. By grafting scions of Green Mountain infected with mild mosaic on Seedling 41956, immune from the latent virus, the virus X was eliminated and the other component was retained. The latter component has been studied on the European varieties President, and Majestic alone and in combination with virus X. This component, free from the virus X, causes a very faint mottling, but a very pronounced mottling and crinkling of the foliage occurs when the virus X is added to it. This mild mosaic virus also has been transmitted by grafting scions of infected Seedling 41956 to 6 Up-to-Date and to 10 British Queen plants. All grafted plants became infected and developed typical top necrosis, manifested by the dying of the top part of the plant, and the development of rather large necrotic blotches on the top and intermediate leaves. No mottling was evident.

Mild mosaic minus virus X has been transmitted to tobacco plants, and 100 per cent infection was often secured when the juice extracted from the leaves of an infected plant was rubbed lightly on the tobacco leaves by means of a cotton swab bearing on the surface a little carborundum dust. The symptoms on tobacco consist of a mottling resembling the mild mottling sometimes produced by the virus X. It was found that it can be readily distinguished from this virus by inoculating infected plants with tobacco mosaic. The tobacco plants infected with virus X, on addition of tobacco mosaic, will invariably develop a spot necrosis on the leaves, whereas the plants infected with the other viruses will develop only tobacco-mosaic symptoms in addition to the mottling resulting from the particular potato virus present. The presence of mild mosaic on tobacco was demonstrated by making return inoculations to Bliss Triumph and Green Mountain, to which the disease was again transmitted. This virus also has been transmitted to *Nicotiana sylvestris* on which it developed fairly distinct symptoms consisting of small, yellow patches following the veinlets.

Crinkle Mosaic

This disease was originally described by Schultz and Folsom (30). It is composed of at least one component in addition to virus X. On the Green Mountain variety it is characterized by a prominent mottling and crinkling of the leaflets. It differs from mild mosaic in that the leaflets are more ruffled and the blotches are larger. This disease also has been studied on the same varieties to which mild mosaic was transmitted, *i.e.*, Burbank, Earliest of All, White Rose, and Bliss Triumph. The symptoms on all these varieties are more intense under the same conditions than those caused by mild mosaic. About the same percentage of this disease is transmitted by leaf mutilation as in the case of mild mosaic. By grafting scions of infected Green Mountain to Seedling 41956, virus X was eliminated and the other component was retained. This also has been studied on the European varieties, President and Majestic, alone and in combination with virus X. As in the case of mild mosaic, the component alone caused a faint mottling, and a much more pronounced mottling and crinkling of the leaves was evident when virus X was added to it. Crinkle mosaic minus virus X produces symptoms on tobacco indistinguishable from those caused by the mild mosaic virus.

Crinkle

This disease is well-known in Europe and has been described as crinkle by Murphy (18), Quanjer (23), and also by Salaman (27), who refers to it as crinkle A, to distinguish it from *para* crinkle. It is composed of at least one virus in addition to X. (Fig. 1.) The presence of virus X was indicated by the interveinal mottle and vein-clearing it produced in *Datura stramonium*, and the ringspot type of mottle on tobacco. The disease has been transmitted to the varieties Green Mountain, Bliss Triumph, Earliest of All and Burbank, in all of which it produced a mottling and crinkling of the leaves very similar to those produced by mild mosaic. By grafting scions of infected President to Seedling 41956, virus X of the complex was eliminated. The remaining component, referred to by Murphy as virus A, has been transferred to President and Majestic, causing a faint type of mottling When virus X was added to it the mottling and crinkling were considerably intensified and were more prominent on President than those caused by mild mosaic. The symptoms of virus A are indistinguishable in tobacco and *Nicotiana sylvestris* from the mild mosaic and crinkle mosaic component free from virus X. (Fig. 2.) Murphy and McKay (18)

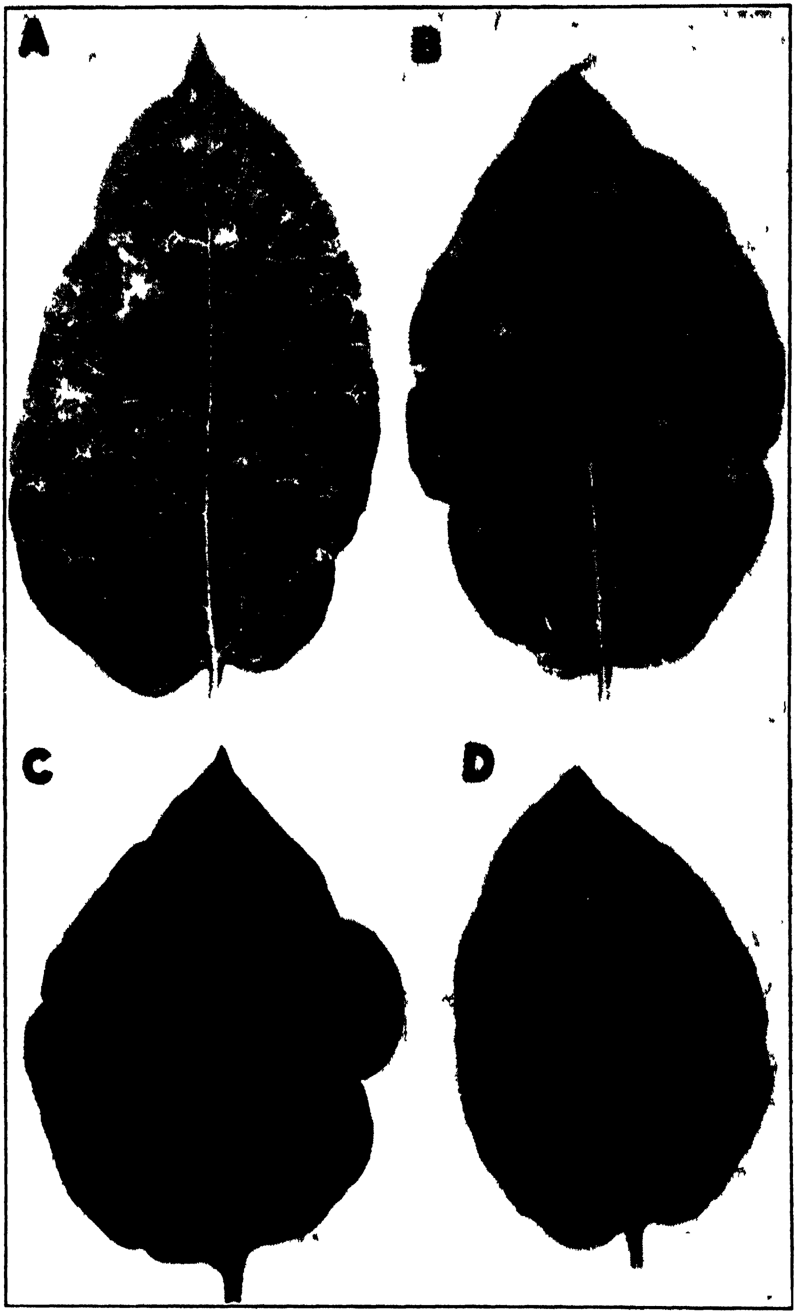

FIG. 1. A and B. Leaflets from Green Mountain. A. Infected with European Crinkle. This virus complex is composed of viruses A and X. B. Infected with mild mosaic. This virus complex is also composed of viruses A and X. C and D. Leaflets from President. C. Infected with Crinkle. D. Infected with mild mosaic.

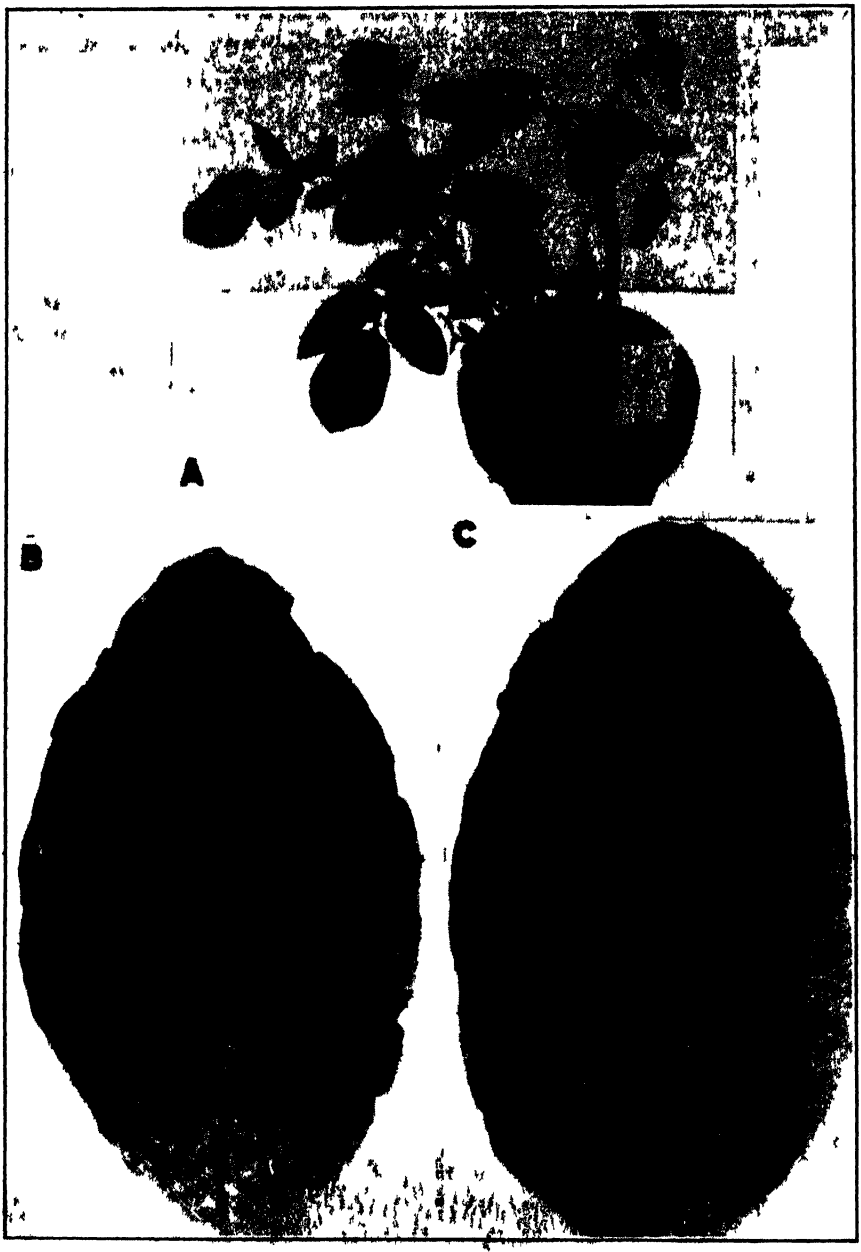

FIG 2. A, British Queen plant showing current season infection of mild mosaic due to the grafting of a scion from seedling 41956 infected with virus A Typical top necrosis, free from mottling. The infected plant was free from virus X B Virus "A" from crinkle on *Nicotiana sylvestris*, due to infecting the plant with juice extracted from an infected 41956 seedling. Notice the clearing of the veins, and the blotchy type of mottling C Virus "A" from mild mosaic on *N sylvestris*, resulting from infection of mild mosaic virus from which the virus "X" had been removed by passing through seedling 41956. Notice the similarity in symptoms caused by virus "A" derived from the European crinkle, and the one derived from American mild mosaic.

showed that virus A produces a necrotic disease on Up-to-Date. Clinch and Loughnane (7) confirmed this, showing that it also produces top necrosis in British Queen. Their results were confirmed when 4 Up-to-Date plants and 4 of the British Queen variety were grafted with scions of Seedling 41956 infected with virus A. All of the plants grafted developed typical top necrosis, showing symptoms very similar to those produced by the transmission of mild mosaic-minus virus X, to the same varieties.

The results obtained on the basis of relative ease of transmission, symptoms produced, and general behavior of mild mosaic, crinkle mosaic, and crinkle indicate that, although the viruses causing these 3 diseases are not identical, they are so similar in their behavior they may be considered as closely related strains of the same virus. It is proposed, therefore, that the viruses causing these 3 diseases be designated as "A".

Leafrolling Mosaic

This disease was originally described by Schultz and Folsom (30). The mottling of the leaflets of Green Mountain plants infected with leafrolling mosaic is diffused and resembles the type found in rugose mosaic. The leaves generally show a rolling upwards, but they are flaccid and resemble the type of rolling found in plants affected with Rhizoctonia or blackleg. This disease also has been studied on other varieties. The rolling and mottling is almost masked in Burbank and Irish Cobbler, but the symptoms in varieties like Earliest of All, Idaho Rural, Bliss Triumph, and White Rose are quite similar to those of Green Mountain. Leaf mutilation has given a fair percentage of infection, about the same as obtained with mild and crinkle mosaic.

Leafrolling mosaic, free from the virus X, as well as the complex leafrolling mosaic and virus X, have been transferred to the English varieties, President and Arran Victory. (Fig. 3.) The symptoms of these diseases on these 2 varieties are characterized by a diffused mottling and rolling of the leaves. The addition or the absence of the latent virus seemed not to influence the symptoms appreciably. All attempts to transmit this virus to tobacco failed. This disease appears to be distinct from any of the other American or European potato viruses that were studied, and it is proposed that the virus causing this disease be designated as virus E.

Para Crinkle

This disease was first described by Salaman and Le Pelley (26). They found this virus universally present in the variety King Edward, apparently a perfect carrier of the virus, as no symptoms can be detected in it. They observed that when this virus was introduced by grafting into Arran Victory it induced a severe disease, but when inoculated by the same method, President failed to produce the slightest reaction. The virus, however, could be recovered from President, by making return grafts to Arran Victory. In a later paper (27), Salaman stated that *para* crinkle

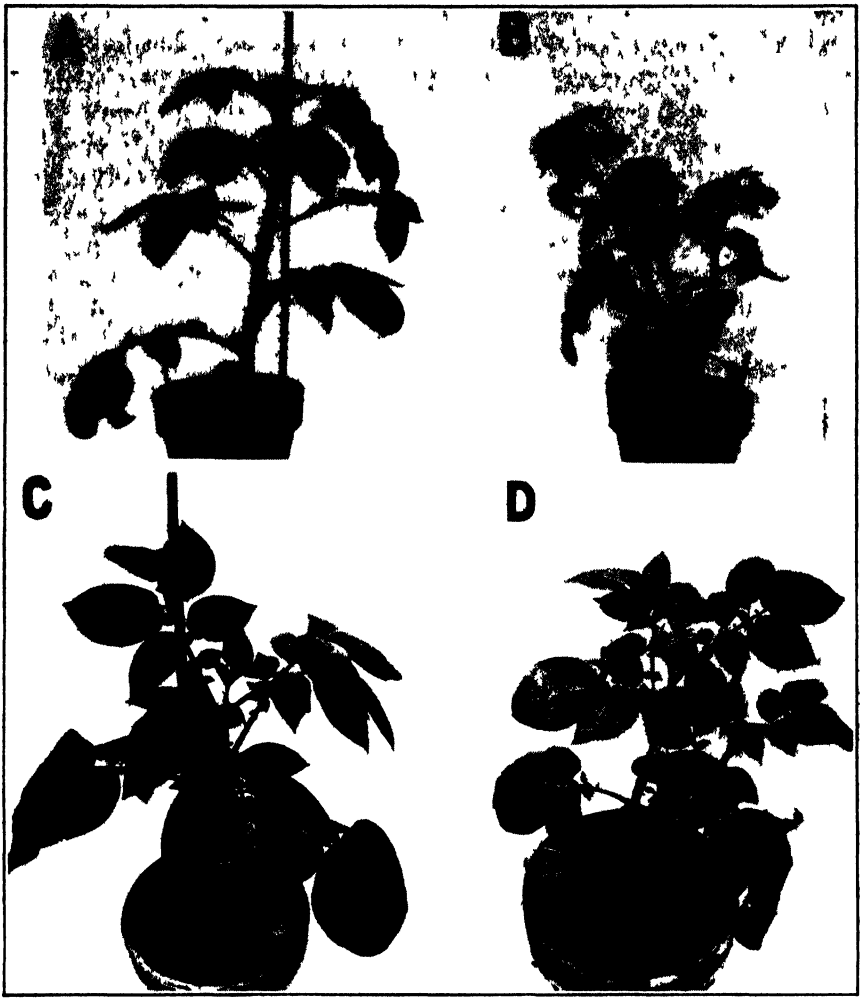

FIG 3. A Healthy Arran Victory B Leafrolling mosaic from which the virus X had been removed was inoculated into Arran Victory Notice the rolling of the leaves, characteristic of this disease. C Healthy Arron Victory. D. *Para* crinkle on Arran Victory. Notice the crinkling and mottling of the leaves.

consisted of a complex of virus Z and virus Y^1, and that passage of the *para*-crinkle complex through a Datura with leaves will break down the complex and remove one of its constituents, namely Y^1. Later, Dr. Salaman told the writer that he no longer held that view, and believed that *para* crinkle was due to the effect of a single virus. We found that *para* crinkle could not be transmitted by juice transfer, but was readily transmitted by stem-grafting. It was transmitted to the varieties Burkank, White Rose, Earliest of All, Bliss Triumph and Green Mountain, in all of which it produced large mosaic-like blotches on leaves, but failed to produce any crinkling or rolling In the variety Burbank, when infected tuber-perpetuated plants were grown under cages in the field, pin-point-like necrotic spots developed in the leaves in addition to the mottling. This disease was also transmitted by grafting to tomatoes and produced a somewhat filiform type of leaf, return grafts from infected tomato to Arran Victory reproduced the disease. Murphy and McKay (19) have suggested that this virus may have affinities with leafrolling mosaic. The writer has studied this disease in comparison with leafrolling mosaic in the American varieties Burbank, White Rose, Earliest of All, and Bliss Triumph, and on the European varieties President and Arran Victory, but no resemblance of the symptoms of these 2 diseases was detected. Whereas leafrolling mosaic can be transmitted by juice transfer, all attempts to transmit *para* crinkle by this method failed.

Veinbanding, Y Virus and Stipple Streak

The rugose mosaic of potato, described by Schultz and Folsom (30), results from a combination of the latent or virus X, and the veinbanding virus. The latter is the aphid-transmitted component of rugose mosaic (14). This virus has been studied in the varieties Green Mountain, Earliest of All, Irish Cobbler, Bliss Triumph, White Rose, and Burbank. There is very little variation in the symptoms produced by it on these different varieties and they consist of small mottled areas grouped close to the main veins. Subjected to high temperature, the mottling may be completely masked, but the crinkling of the leaves and their tendency to curl slightly downward persist. Some of the veins of the lower leaves generally are necrotic, resembling black pencil-like lines. The affected plants are noticeably stunted and generally die prematurely. In contrast with most of the other potato viruses, the symptoms attributable to current season infection differ from those produced in succeeding years because of tuber perpetuation of the disease. (Fig. 4.) In many varieties they become manifest as a burning or necrosis of the veins of the newly developed leaves and a dying of the tissue between the veins. These leaves eventually will drop, or hang by a thread of tissue to the stem. In the European variety President it produces a mottling in the top leaves and some necrosis, especially in the intermediate leaves; in the second generation only a diffused mottling of the leaves was evident, but almost no necrosis of the veins, or leaf drop. In Arran Victory there was a diffused mottling, but neither necrosis nor leaf drop. Only a diffused mottling was noticed in infected Epicure. In tobacco this virus produces a banding of the veins, and, during later stages of the disease, a general mottling develops on the leaves; in tomato no symptoms were evident other than a faint clearing of the veins. This virus also produced a banding of the veins in the pepper, later changing to a mottling. In infected petunia plants a faint vein clearing of the leaves was evident, and distinct mottling developed in the petals.

Smith (31) originally described virus Y as one of the commonest and most destructive of potato viruses in England. The symptoms caused by

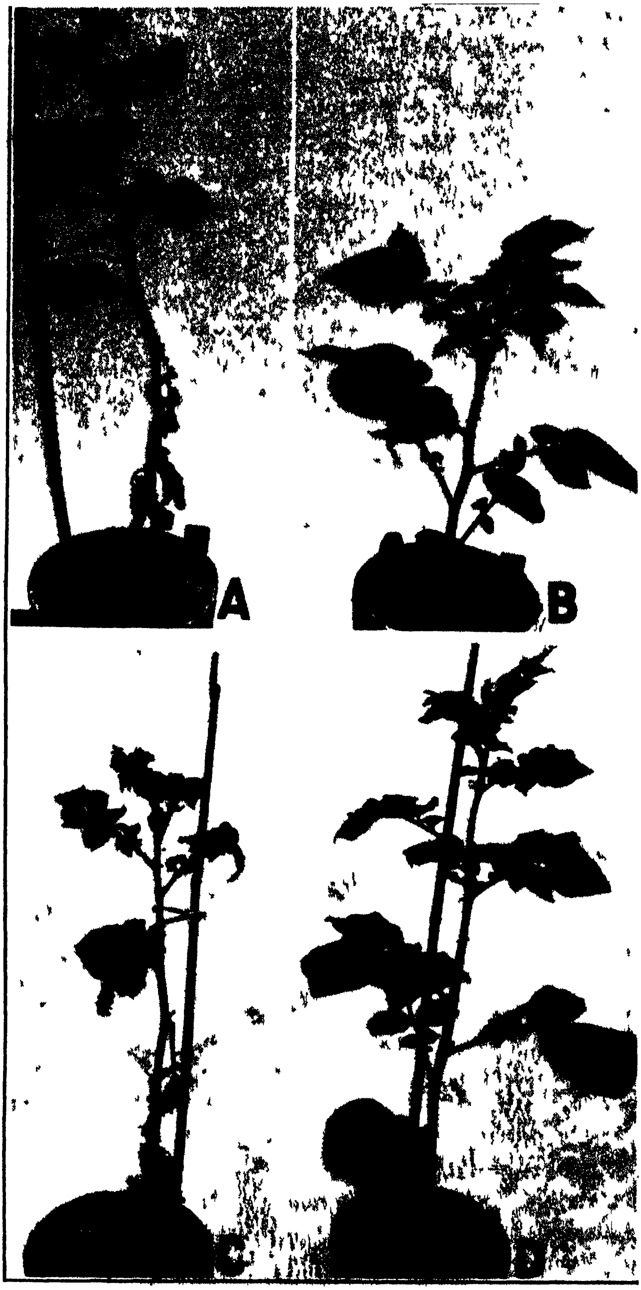

FIG 4. A and B. President plants. A. Free from the virus X showing tuber-perpetuated symptoms of the virus Y. Notice the leaf dropping. B. Showing tuber-perpetuated symptoms of the veinbanding mosaic. The veins of the lower leaves are necrotic, but no leafdrop is evident. C and D. White Rose potato plants. C. Showing tuber-perpetuated symptoms of virus Y. The White Rose also carries the virus X. D. Showing tuber perpetuated symptoms of veinbanding mosaic. Since this variety also carries the virus X, the disease caused by the virus complex is known as rugose mosaic.

it have been studied in a number of American and European potato varieties. After an incubation period of 15 to 20 days a blotchy mottle appears in the variety President, spreading from the veins and affecting only the uppermost leaves. This mottle later becomes intensified and is accompanied by some wrinkling and waviness of the leaves. A little later, necrosis appears on the under sides of the veins of leaves occupying an intermediate position on the stem. These necroses increase in severity and spread most rapidly along the course of the veins on the under surface of the leaf, appearing as elongated brown stripes on the petioles and as blotches between veins. The necroses pass down the petiole to the main stem and the leaf then collapses, rapidly withers, and remains hanging by a thread of tissue. The stem often shows elongated brown stripes. In Arran Victory and Epicure the symptoms are those of only a mild mottling. The current season symptoms of virus Y in Irish Cobbler, White Rose, Burbank, Bliss Triumph, Green Mountain, and Earliest of All, which also harbor virus X, are manifested by necrotic leafspots including the veins and the tissue immediately surrounding them. Nearly every leaf on the plant may eventually drop but remain hanging on the main stem by a thread of tissue, leaving only a tuft of leaves at the tip of the plant. Plants from tubers of plants infected with virus Y appear at first as if infected with rugose mosaic, except that the necrosis of the veins is found in almost every leaf instead of being confined to the lower leaves. While the plant is still immature, the lower leaves, at first green, gradually turn yellow and drop but remain hanging on the stem by a thread of tissue. In the variety Bliss Triumph the petioles develop necrotic spots and stripes of streak are found on the stem. Finally, all the lower leaves dry down and hang on the main stem. At this stage of development the disease appears to be quite different from rugose mosaic. These observations were made also by Koch and Johnson (15) who state: "It appears certain, therefore, that Smith's virus Y, while possessing many characters in common with the American veinbanding virus, is not identical with it."

The virus Y in tobacco causes a distinct banding of the veins, followed later by a general yellowing between the veins. The symptoms are much more pronounced than those caused by the veinbanding mosaic. Inoculations into petunia failed to cause a mottling of the petals, whereas veinbanding and stipple-streak virus generally produced mottling. On pepper the symptoms were quite similar in pattern to those produced by the veinbanding virus, except that they were more pronounced.

In order to determine whether or not virus Y has any effect upon potato plants infected with rugose mosaic the following experiment was started in the spring of 1935. Each of 6 tubers of the variety Green Mountain and 6 of Bliss Triumph infected with rugose mosaic were cut into three pieces. Each seed piece was planted in a 6-in. pot in the greenhouse. When the plants were about 3 or 4 inches high, those derived from one seed piece of each tuber were inoculated with virus Y by the rubbing

TABLE 1.—*A summary of comparison of the properties of the Y group viruses*

Viruses derived from	Thermal inactivation point °C.	Number infected	Tolerance to dilution	Number infected	Longevity *in vitro* 1–10 at 15° C.	Number infected	Resistance to pH, using disodium phosphate-citric acid buffer, after 24 hours	Number infected
					Hours			
Y virus from England	50	10[a]/10[b]	1–10	10/10		10/10	2.2	10/0
			1–100	10/8	24		3.8	10/0
	54	10/7	1–200	10/8			4.2	10/1
			1–300	10/6	48	10/9	4.7	10/5
	56	10/2	1–400	10/8			5.	10/5
			1–500	10/5	72	10/3	5.6	10/9
	57	10/0	1–600	10/2			6.	10/8
			1–700	10/2	96	10/0	7.	10/6
	59	10/0	1–800	10/0			8.5	10/4
			1–900	10/0			1 − 10	10/10
	61	10/0	1–1000	10/1			H_2O	
	Untreated Control	10/10						
Stipple streak from The Netherlands	50	10/10	1–10	10/10	24	10/9	2.2	10/0
			1–100	10/10			3.8	10/0
	54	10/1	1–200	10/9	48	10/8	4.2	10/0
	56	10/0	1–300	10/6			4.7	10/2
	57	10/0	1–400	10/10	72	10/2	5.	10/3
	59	10/0	1–500	10/8			5.6	10/8
	61	10/0	1–600	10/8	96	10/0	6.	10/6
	Untreated Control	10/9	1–700	10/8			7.	10/6
			1–800	10/6			8.5	10/2
			1–1000	10/4			1 + 10	10/9
				10/2			H_2O	
Vein-banding virus from North America	50	10/8	1–100	10/10	24	10/9	2.2	10/0
			1–100	10/6			3.8	10/0
	54	10/2	1–200	10/4	48	10/8	4.2	10/1
			1–300	10/2			4.7	10/3
	56	10/4	1–400	10/6	72	10/2	5.	10/5
		10/0	1–500	10/0			5.6	10/9
	57	10/0	1–600	10/4	96	10/0	6.	10/6
			1–700	10/4			7.	10/7
	59	10/0	1–800	10/0			8.5	10/6
	61		1–900	10/2			1 − 10	10/10
	Untreated Control	10/10	1–1000	10/0			H_2O	

[a] Numerator: number of plants inoculated.
[b] Denominator: number infected.

method. Plants of a second duplicate series were inoculated with the veinbanding virus, and the third, or control, series was not inoculated. These plants were kept under observation for more than two months after inoculation, but none of them developed symptoms different from the controls, which showed the typical tuber-perpetuated rugose mosaic symptoms. This experiment indicated that the veinbanding mosaic in the rugose-mosaic complex had a protective effect that prevented the development of symptoms typical of virus Y in Bliss Triumph.

The stipple-streak virus was first described by Atanasoff (1) and is universally present in the Dutch variety Zeeland Blue. Although the virus is masked under high temperature, this variety cannot be considered as a carrier because a definite interveinal type of mottling is evident under low-temperature conditions. In addition to the stipple-streak virus, the tubers of Zeeland Blue, received from Holland, also contained a weak strain of virus X. Murphy and Loughnane (21) state: "It was formerly thought that the responsible virus in this material was virus Y, but more than one virus is present, and the analysis is not yet complete." Since Zeeland Blue has been grown as a commercial crop in Holland, it undoubtedly has contracted other viruses. The writer, however, believes that the typical symptoms in potatoes and other solanaceous plants caused by stipple streak are, primarily, caused by the stipple-streak component and not by a complex. In our tests with this virus, the X component was removed by inoculating juice from an infected Zeeland Blue into Seedling variety 41956.

In many potato varieties circular necrotic spots developed in the inoculated leaves; and necrotic spots, rather than burning of the veins, were found in the intermediate non-inoculated leaves.

The stipple-streak virus causes a banding of veins in tobacco leaves, but the symptoms are more like those caused by the veinbanding virus than virus Y. (Fig. 5.) In *Nicotiana sylvestris* a definite veinbanding is observed, but the band following the veinlets is thinner than that caused by virus Y. Infected plants show less rugosity than when the infection is veinbanding. The symptoms in petunia were rather like those produced by the veinbanding mosaic, and a mottling of the petals of infected plants was evident.

Virus Y was inoculated into 10 Zeeland Blue plants carrying the stipple-streak virus. These plants were kept under observation for a period of 10 weeks, but no additional symptoms attributable to the virus Y were evident. When, at the end of that period, juice from leaves of each of these 10 plants was inoculated into 20 tobacco plants, only the faint veinbanding characteristic of the stipple-streak virus appeared. This showed that this virus protected the plants from infection with virus Y.

The symptoms produced by the Y, veinbanding, and stipple-streak viruses vary in degree and intensity in a given potato variety, but are similar in type. In some varieties a mottling develops; in others an acropetal necrosis, but no instances have been observed where one of these viruses caused a mottling and another an acropetal necrosis, or *vice versa,* in the same variety. The same general symptoms also have been observed in other solanaceous plants, but, in all cases, the effect of the virus Y was the most severe. K. Starr Chester, as a result of serological studies, reported in a letter dated May 29, 1937: "The European strain of Y virus proves to be serologically indistinguishable from our ordinary veinbanding virus. Stipple-streak also belongs to the veinbanding group." Dilution test, thermal-inactivation-point determination, longevity in vitro, the effect of pH on the viruses (Table 1), protective inoculation studies, and serological investigations, all tend to prove that these 3 viruses are closely related strains of the same virus. It is proposed, therefore, to include these 3 strains under the designation virus Y.

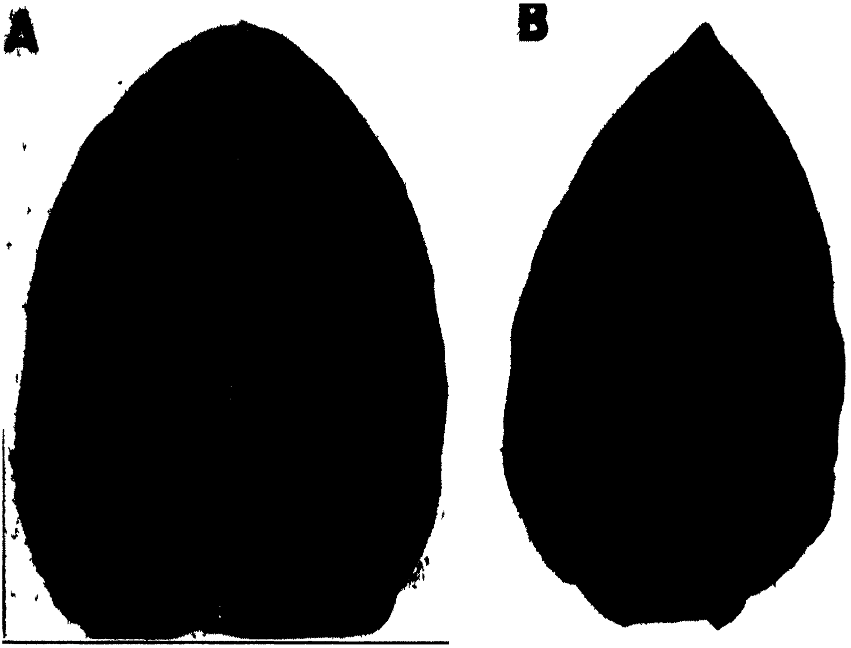

FIG. 5. A. Tobacco leaf infected with veinbanding mosaic. B. Tobacco leaf infected with the virus Y, notice that the symptoms are of the same general type, but Y causes a more pronounced clearing of the veins than the veinbanding mosaic.

Chester (6) states, as a result of his serological studies with different viruses, that such a close serological relationship is shown between the veinbanding virus of potato and cucumber mosaic one may be well justified in regarding these as strains of the same virus type. This heretofore unrecognized relationship is further borne out by the fact that potato veinbanding and cucumber mosaic viruses each produce in the cowpea, *Vigna sinesis* (L.) Endl., local brown necrotic lesions that are indistinguishable.

To determine whether cucumber mosaic could be transmitted to potato, 40 potato plants of Green Mountain, Bliss Triumph and some seedling varieties were inoculated in 1936 with extracted juice from infected cucumber plants. None of these plants developed symptoms, nor was it possible to recover the virus from inoculated plants by making return inoculations to tobacco.

The tubers from the inoculated plants were saved and 35 of them were planted in the greenhouse in the fall of 1937. All of the plants from these tubers appeared healthy, and, when inoculated with the veinbanding virus, developed symptoms typical of this disease, which indicated that no virus was present in these plants to protect them against veinbanding virus infection. Ten potato plants were grafted with scions of cucumber-mosaic-infected tobacco plants, but these, also, failed to produce any symptoms. Three series of 10 tobacco plants were inoculated, respectively, with veinbanding virus, virus Y, and stipple streak. After symptoms had developed in these plants, they, as well as 10 healthy control plants, were inoculated with cucumber mosaic. All plants showed typical cucumber-mosaic symptoms, and there was almost no difference in the time required for symptoms to develop in the 4 series of tobacco plants, which showed that no protection against infection of the cucumber-mosaic virus was afforded by the presence of the veinbanding, the Y, or the stipple-streak virus. On the basis of our inoculation studies, we must conclude that there is no relationship between cucumber mosaic and potato veinbanding.

The Potato Mottle Virus or Virus X

In 1925 Johnson [illegible]) described the "mottle virus," which was shown to be regularly present in masked form in all tubers of most, if not all, standard varieties of "apparently healthy" potatoes. In Europe this disease, which shows a mild type of mottling in some varieties, has been described as simple mosaic (19). According to Murphy and McKay (19), it corresponds exactly to "common mosaic," as described by Quanjer (23), and is identical with the "Arran Victory mosaic," of the Cambridge workers. Murphy (19) states that "one of the latent viruses, commonly present in the American material, both diseased and healthy, was a necrotic form, identical with the common streak of Up-to-Date and other similar sorts." Murphy apparently referred to the virus B, which will be considered later. He goes on to state "another virus which, according to our experience, was even more common in this material, was European simple mosaic. Certain American "healthy" plants were found which were apparently free from streak, but no such plants were free from simple mosaic." Kenneth Smith (31) designated the virus causing this disease as X. This virus is not always found in European varieties, although the writer, when in Europe during the summer of 1935, was informed by different investigators, that virus X is quite common in nearly all varieties, and as much as 40 or 100 per cent of some varieties may be infected with it.

The symptoms caused by virus X in Datura and tobacco are so well known that it is not necessary to discuss them in detail. The writer has transmitted this virus also to *Amaranthus retroflexus,* in which it produced

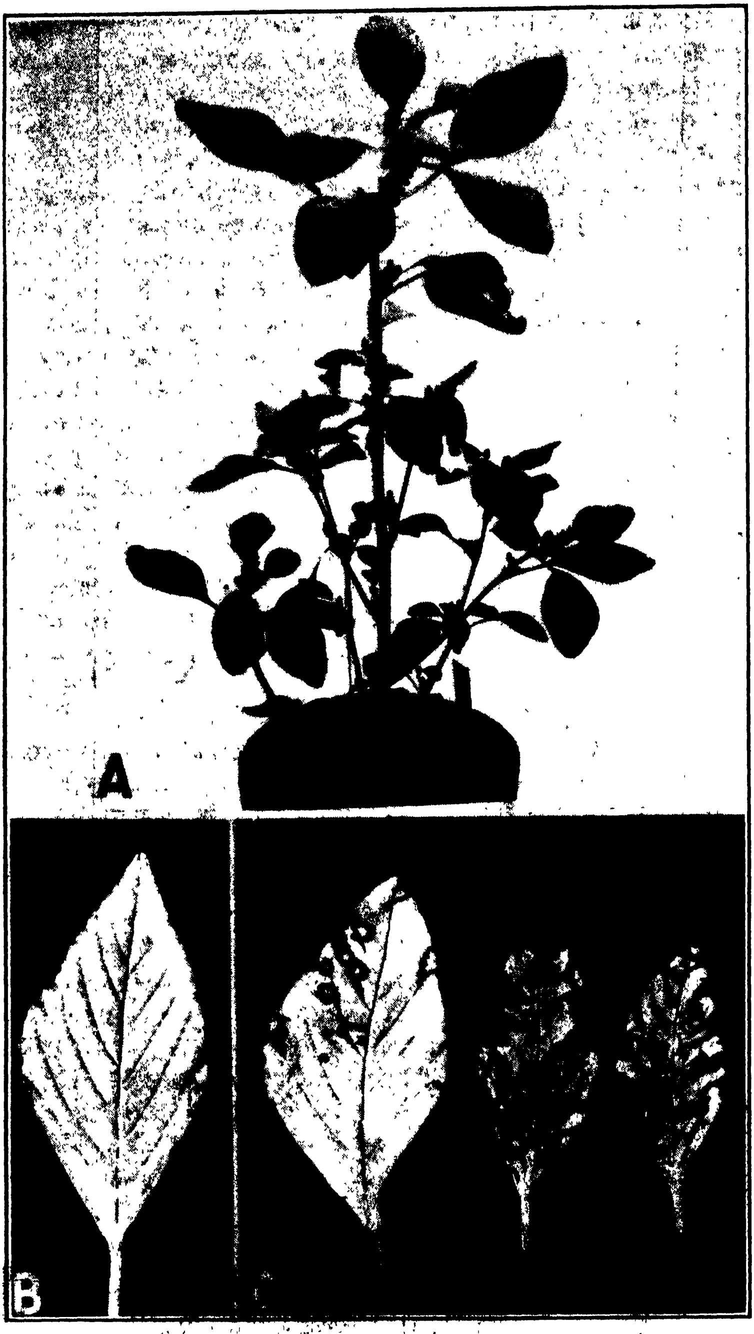

FIG. 6. A. Pigweed, *Amaranthus retroflexus*, infected with virus X, due to inoculating the plant with juice from a potato plant containing this virus. The presence of X was demonstrated by making return inoculations to Jimson weed in which the characteristic X symptoms developed. B. Leaflet from healthy pigweed. C. Leaflets from pigweed, showing necrotic blotches due to infection of virus X.

necrotic spots on the leaves (Fig. 6). The virus was readily recovered from infected plants by making return inoculations to *Datura stramonium,* in which it produced the typical X type of mottle. This is believed to be the only potato virus that has been successfully transmitted to plants outside the Solanaceae.

It was interesting to note that Seedling 41956 was immune from all strains of virus X, with which the writer has been working. Bawden (3) described virus D, which he places in or near the X group on account of the possession of a number of properties characteristic of X. Since this virus appeared to be so different from the other strains of X, a tuber infected with virus D received from Bawden was planted and the characteristics of this virus were studied. Observations similar to those of Bawden were made in our experiments, namely, that almost no symptoms are produced by this virus in Datura and tobacco, but that a severe acropetal necrosis develops in Arran Victory and President when infected with D (3), whereas X develops only a faint mottling. It was not possible to transmit this virus D to Bliss Triumph, Green Mountain, and Irish Cobbler. This was to be expected, since these varieties are already carrying X, which, therefore, would protect the plant against infection of any other strain of the same virus type. Necrotic spots, however, were produced in healthy Green Mountain seedling varieties which were free from the virus X.

Every attempt to transmit virus D to Seedling 41956, either by leaf mutilation or by side grafting, failed, as was demonstrated by the failure to recover the virus by subsequent inoculations to pepper, which indicated that this variety is resistant to all strains of virus X that have been tested.

Virus B

Murphy and McKay (19), reported that in their study on the comparison of some of the European and American viroses of the potato they had considerable difficulty in introducing certain American viruses into healthy President plants. Throughout the American material there was found a latent virus or viruses that had a severe necrotic effect on President. This latent virus was found both in the "healthy" Green Mountain and in the obviously diseased plants. The writer has grafted scions of Earliest of All and Bliss Triumph that were infected with mild mosaic, crinkle mosaic, and leafrolling mosaic, on healthy Arran Victory and President. In many cases top necrosis developed on these 2 varieties. Scions from commercially healthy Green Mountains almost invariably developed similar symptoms, whereas they were produced only occasionally when scions from Burbank, Earliest of All, or Bliss Triumph were used.

These top-necrosis symptoms could not be ascribed to the so-called latent mosaic or virus X, which is universally present in healthy American commercial potatoes, because this virus produces in Arran Victory and President only a mosaic type of mottling without necrosis. (Fig. 7.)

Grafts from the English variety Up-to-Date, which, according to Bawden, carries virus B and virus X, produced typical top necrosis in President and Arran Victory It was considered of interest to determine whether or not the combination of B and X is necessary to produce this disease. Accordingly, double grafts were made of scions from Up-to-Date on healthy scions of virus X resistant Seedling 41956, which were in turn grafted on healthy Arran Victory Typical top necrosis developed from virus B, since virus X was filtered out of the complex, as was proved by subsequent inoculation of juice from the infected Arran Victory plants onto Datura and pepper, which failed to show the presence of X. This demonstrated definitely that virus B alone was responsible for the top necrosis. Similar double grafts were made, but, instead of using Up-to-Date, scions from commercially healthy Green Mountains were used. Again, in these cases top

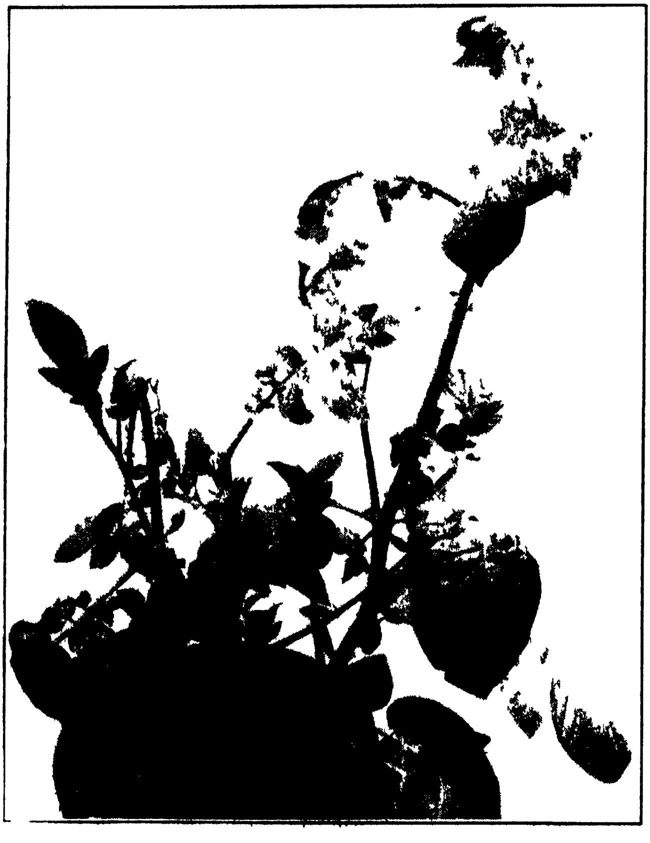

FIG. 7. Arran Victory plant showing top necrosis due to infection of virus B alone. A scion from Up to Date carrying both X and B viruses was grafted onto X resistant seedling 41956 The virus X was removed by this seedling variety, and a scion from it containing only the virus B was grafted onto a healthy tomato plant, which failed to develop any symptoms. When a scion from this plant was grafted onto Arran Victory typical virus B symptoms, namely top necrosis, developed. Subsequent inoculations from this plant to Jimson weed and pepper failed to show the presence of the virus X.

necrosis developed in Arran Victory, and subsequent subinoculations to Datura and pepper demonstrated the absence of virus X.

Since it is known that the Katahdin variety produces a severe top necrosis when grafted with scions of Green Mountain, the question was raised whether this symptom is produced by virus X or by virus B. Grafts from Arran Victory, infected only with virus B and showing current-season symptoms of this disease were made on Katahdin. In all cases necrotic circular and rectangular spots developed on the intermediate leaves first, and gradually became evident on every leaf of the plant, except the very youngest. Double grafts of Green Mountain on Seedling 41956 and Katahdin, as previously described, also were made. In these cases a top necrosis developed, consisting of a necrotic spotting of the top leaves, and a slight necrosis of the terminal end of the stem. The necrotic spots were larger and not nearly so numerous as those produced by virus X alone, and the top part of the plant did not die, as it did in the case of infection by X. About 40 tubers of Green Mountain grown in Maine were taken at random and every one was found to be infected with virus B. It appears, therefore, that most of the commercially healthy Green Mountain plants contain virus B in addition to virus X. Tests made of more than 100 tubers of Burbank, Earliest of All, and Bliss Triumph varieties grown in Oregon failed, with two exceptions, to show the presence of virus B. The fact that Burbank, Earliest of All, and Bliss Triumph, infected with different mosaic viruses, produced top necrosis when grafted on Arran Victory and President may be explained by the fact that these plants had become infected by core grafts from mosaic-infected Green Mountain; and, by this method, virus B, in addition to the mosaic, may have been transmitted from the carrier, Green Mountain.

Grafts of scions from Seedling 41956 infected with virus B alone were made on tomato plants. No symptoms of any kind were discernible, but when scions from these tomatoes were grafted on Arran Victory, typical top necrosis resulted indicating that the tomato is a host of virus B, although it fails to show any symptoms.

It is not known what effect virus B has on plants that are merely carriers of it. It does not appear to be so infectious as the virus X, since it is not transmissible to potato by leaf mutilation.

Di Vernon Top Necrosis

This virus has been described by Bawden (according to Smith (32, p. 317) who refers to the component causing top necrosis in certain potato varieties as virus C. In addition to this, virus X also is present.

In the fall of 1934 several plants of the varieties Burbank, Bliss Triumph, and Earliest of All were grafted with scions from infected Di Vernon plants. In nearly all cases current-season symptoms developed that were characterized in these three varieties by a severe top necrosis, consisting of a streaking of the stem and petioles and by numerous small circular necrotic spots on the foliage.

The second-generation symptoms of this disease in these varieties, as observed in plants growing under cages in 1935, were manifested by a slight mottling of the foliage without any indications of necrosis. Scions from potato plants showing second-generation symptoms were grafted on Burbank, Earliest of All, and Bliss Triumph. In nearly all cases a mottling developed in the grafted plants and in no case was top necrosis evident. This behavior raised the interesting question, whether the top-necrosis virus was destroyed and another unknown component of the complex was responsible for the mottling, or whether the passage of the virus through plants in which it caused severe necrosis had caused the virus to become attenuated. If the virus had become attenuated it was believed it would still protect infected plants from developing top necrosis when grafted with scions from Di Vernon. Accordingly, 47 plants showing second-generation symptoms were grafted with scions from Di Vernon; of this number 6 developed top necrosis, and the remaining 41 failed to develop any additional symptoms. This indicated protection, since a very high percentage of infection was always obtained when healthy plants were grafted with this virus. The same observation, namely, that an initial top necrosis, presumably caused by virus B, became a nonnecrotic mosaic in the variety President, has been described by Oortwyn Botjes (5), and this effect was attributed by him to attenuation.

This virus complex has been transmitted by leaf rubbing to tobacco, in which the symptoms developed were very similar to those produced by the rugose-mosaic complex, *i.e.*, a severe spot necrosis.

Grafts from such infected tobacco plants to potato plants failed to infect the latter. Since the symptoms on tobacco seemed to indicate that one of the components of the complex might be quite similar to the veinbanding mosaic, an effort was made to determine whether plants infected with the Di Vernon complex protects against either the Y or veinbanding virus. Accordingly, 5 potato plants showing second-generation symptoms of this virus complex were inoculated with the virus Y and every one of them became infected. Ten infected potato plants were inoculated with the veinbanding virus, and every one of these also showed the presence of this virus. These results would indicate that the virus Y group is not related to any of the viruses found in the Di Vernon complex.

Double grafts of scions of Di Vernon on Seedling 41956, and then on President, developed typical top necrosis on the latter. Since the Seedling 41956 filters out the virus X, and since the absence of this virus in the infected President variety was indicated by subsequent inoculations to pepper, it was proved that the virus X component is not necessary to produce top-necrosis symptoms.

Scions from a Green Mountain plant showing current-season symptoms of top necrosis were grafted on President and on healthy Green Mountain seedlings. Circular necrotic spots developed in the intermediate leaves, but not in the young top foliage. This seemed to indicate that acronecrosis develops only when a carrier of virus C, like Di Vernon, is grafted on a susceptible potato plant, whereas a scion from a plant showing top necrosis due to current season infection will produce only acropetal necrosis. Tubers from plants having top necrosis as current symptoms of C virus infection give rise to plants, which, when subsequently grafted on susceptible plants, show only a mottling, and have lost the power to produce top necrosis.

Although the symptoms in tobacco produced by this virus complex suggests the presence of a Y-like virus, all other evidence indicates that none of the components of the virus complex belongs to the Y virus group. Whereas the Y-like viruses are readily transmitted to potato by leaf mutilation, all attempts to transmit to this host the virus components in the extracted juice of Di Vernon plants failed to produce any symptoms. The Y-virus group produces acropetal necrosis on potatoes, whereas the Di Vernon virus complex causes a definite acronecrosis as a current-season symptom in the same varieties. It is believed that the component causing top necrosis differs from any of the potato viruses found in America.

DISCUSSION

An attempt has been made to determine the identity of the viruses affecting potatoes in Europe and in this country. The latent mosaic of healthy potatoes, or virus X, is universally present in all the older commercial varieties grown in the United States, so that all the viroses affecting those varieties are attributable to combinations of 2 or more viruses. The development of Seedling 41956 by the United States Department of Agriculture, found to be immune from the virus X, has been of considerable aid in isolating the individual viruses of virus complexes. A number of the European viroses were found traceable also to complexes including X, but in some cases they were caused by only one virus.

The symptoms caused by the different viruses, individually, and by the virus complexes have been studied in several different American and European potato varieties, and also in other solanaceous plants. In addition the longevity of the virus outside the host, the effect of dilution of the virus, and its reaction to various pH concentrations have been determined in some cases. Serological determination and protective-inoculation studies also have been employed to determine the relationship between different viruses.

On the basis of symptoms and other characters it is believed that enough evidence has been obtained to show that crinkle, mild mosaic, and crinkle mosaic are very similar, and that they may be considered as closely related, although not identical, diseases. All 3 are caused by 2 virus components. Crinkle contains, in addition to virus X, the virus designated by Murphy (18) as A. It is suggested that the virus component in mild mosaic and crinkle mosaic, in addition to X, be designated also as virus A.

The leafrolling-mosaic virus appeared to be different from any of the known viruses occurring in potatoes in Europe. It has been suggested by Murphy (19) that this virus may have affinities with the *para* crinkle virus. These 2 diseases have been studied in comparison in several different American and European potato varieties, but no similarity of symptoms or of any other characteristics were found in any of them. Whereas leafrolling mosaic manifested itself in most of the varieties by a soft upward rolling of the leaves in addition to a diffused mottling, *para* crinkle failed to produce a rolling and developed large mosaic-like blotches on the leaves of most varieties. Leafrolling mosaic can be transmitted by juice transfer, whereas all attempts to transmit *para* crinkle by this method failed.

The absence or presence of virus X in the leafrolling-mosaic complex did not appear to effect any significant difference in the development of symptoms in the varieties President and Arran Victory. It is proposed to designate as E the virus causing leafrolling mosaic.

The veinbanding, Y, and stipple-streak viruses caused symptoms in different potato varieties and other solanaceous plants that varied in intensity, but were quite similar in general appearance. Of these 3 viruses the stipple-streak and veinbanding appear to be more similar to each other than the virus Y is to either one of them; the last-named is considerably more severe in its attack. Both the veinbanding and stipple-streak viruses, when present in potato and tobacco, protected against infection by virus Y. Serological studies by Chester showed that these 3 viruses are serologically indistinguishable. There were no appreciable differences in the thermal inactivation point, tolerance to dilution, longevity *in vitro,* and resistance to pH concentrations, for the 3 viruses. It was of interest to note that this virus group was readily inactivated at a pH of less than 4. Our findings on the properties of these viruses are in general agreement with those reported by Koch and Johnson (15).

Different strains of virus X have been found in some of the European and American potato varieties. The intensity of symptoms of such complexes causing crinkle and mild mosaic varied somewhat, depending upon the strain of X present in the complex. This virus has been successfully transmitted from potato to *Amaranthus retroflexus,* on which it produced necrotic spots in the leaves. The virus D described by Bawden (3) is an aberrant strain of X, as was intimated by him. It is believed that this virus also should be designated as X instead of D. Seedling 41956 was found to be immune from all strains of virus X tested.

Virus B, universally present in combination with X in the European variety Up-to-Date, which serves as a masked carrier of both of these viruses, was obtained in biologically pure form by making double grafts of scions of Up-to-Date onto Seedling 41956 and Arran Victory. Virus B alone produced typical top necrosis on the latter variety. The same results were obtained when commercially healthy Green Mountain tubers from Maine instead of Up-to-Date, were used; whereas more than 100 tubers comprising Burbank, Earliest of All, and Bliss Triumph grown in Oregon, failed, with 2 exceptions, to show the presence of virus B. Samples of several potato varieties selected from different States should be tested to determine how generally this virus is distributed and what effect, if any, it has on potatoes that act as carriers of it.

Virus C, which, according to Salaman (27), is universally present in the variety Di Vernon, was readily transmitted by grafting to all of the American varieties tested, and produced severe top necrosis as current-season symptoms. Second-generation symptoms, however, consisted of normal-size plants, showing a distinct but faint mottling. Grafts of scions from such plants on different susceptible varieties produced only a mottling instead of top necrosis. Studies are in progress to determine whether this is caused by the attenuation or by the disappearance of one of the virus components of the complex.

Virus C, alone, produced a vein-clearing on tobacco plants, and viruses C and X, together, caused typical spot necrosis on this host, indistinguishable from the symptoms produced by rugose mosaic. Subsequent studies failed to show any relationship between the viruses C and Y.

SUMMARY

The chief symptoms, as found on different American and European potato varieties and certain other solanaceous plants, are described for the following viroses: mild mosaic, crinkle mosaic, crinkle, leafrolling mosaic, and *para* crinkle, and the veinbanding, Y, stipple streak, X, top-necrosis B, and top-necrosis C viruses.

The relationship existing between different viruses has been determined on the basis of (a) symptoms on different host plants, (b) protective inoculation, (c) serological reactions, and (d) physical properties, such as longevity *in vitro*, tolerance to dilution, and thermal inactivation.

Mild mosaic, crinkle mosaic, and crinkle, although not identical, were found to be so similar in many respects that the virus, in addition to X, found in the complex causing each of the 3 diseases, is designated as virus A.

Leafrolling mosaic was found to be distinct from *para* crinkle. The virus in addition to virus X in this complex, is given the new designation E.

Veinbanding, Y, and stipple streak viruses vary somewhat in severity in different hosts, but the general type of symptoms is similar. Serological and property studies prove that they are closely related strains of the same virus, and all 3 are here included under virus Y.

No relationship was found between the veinbanding and the cucumber-mosaic viruses on the basis of inocluations of potato and tobacco. None of the virus Y strains protected against infection with cucumber mosaic on tobacco.

Amaranthus retroflexus proved to be a host of virus X. Virus D, of Bawden, was shown to be an aberrant strain of virus X. Seedling 41956 was immune from all strains of virus X tested.

Virus B produced top necrosis in the varieties Arran Victory and President. Green Mountain was found to be normally a symptomless carrier of this virus in addition to virus X. It was found only occasionally in the varieties Bliss Triumph, Burbank, and Earliest of All, grown on the Pacific coast.

Virus C causes top necrosis, as a current-season symptom in all of the American potato varieties tested. The tuber-perpetuated symptoms in these varieties consist of a mottling, only. Grafts of scions from the latter plants to healthy potatoes produce a mottling instead of a top necrosis.

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SCOLECOSPORES IN DIPLODIA ZEAE[1]

HELEN JOHANN[2]

(Accepted for publication Aug. 30, 1938)

Within the past year scolecospores have appeared in cultures of one strain of *Diplodia zeae* (Schw.) Lév. isolated from rotted corn kernels obtained from Ohio in the spring of 1934 and maintained in the laboratory at Madison, Wisconsin, since that time. Mycelial transfers and single-spore cultures had been made from time to time, but not until 1937 were scolecospores observed. They were noted first along the line of contact of 2 strains of *D. zeae* on a 6-week-old plate of synthetic agar (Fig. 1, *A*). Macroscopically, the discharged spores appeared as small bead-like masses among the pycnidia located along the aversion line some time after the dark brown pycnospores were mature. Later, they were produced on several different agars.

More recently still, they have been found in a plate containing several mass transfers from colonies that were growing out of kernels from an ear inoculated with this strain of *Diplodia* in the field at Madison, Wisconsin, in 1937. The reisolated colonies had the appearance of physiological old age. The mycelium browned early and numerous dark erumpent fruiting bodies appeared on the surface of the agar. No tan-color masses of scolecospores were observed. However, when the fruiting bodies were crushed, both pycnospores and scolecospores were present on the slide. It thus seems that scolecospores may be formed in a culture without their presence being visible macroscopically.

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[2] The writer is indebted to Eugene H. Herrling, Department of Plant Pathology, University of Wisconsin, for the photographs.

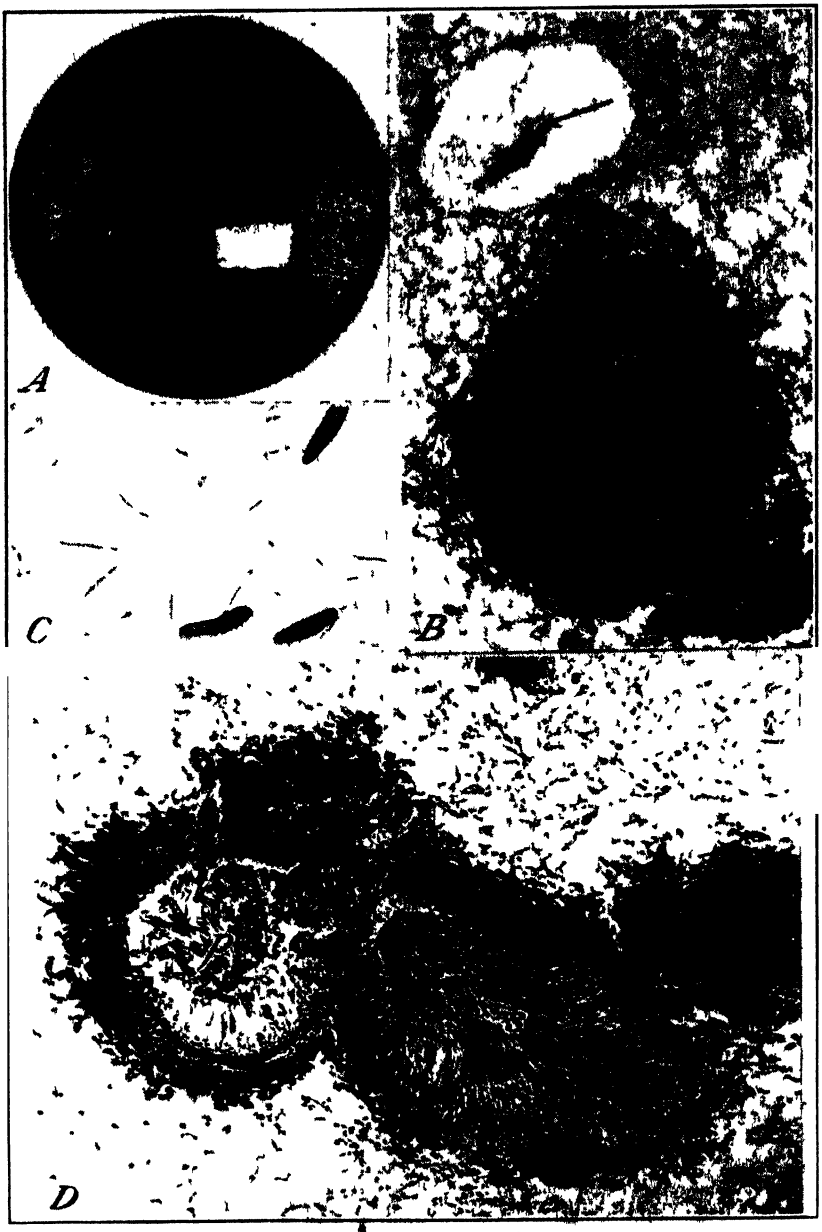

FIG. 1. Photomicrographs of spores and fruiting bodies produced in plates of synthetic agar on which two strains of *Diplodia zeae* had been planted. The fruiting bodies containing scolecospores were located along the line of contact of the two colonies The submerged pycnidia were sectioned parallel to the surface of the agar; those exposed, at right angles to the surface. The sections were stained with Flemming's triple stain *A*. Lower side of plate containing two colonies of *Diplodia zeae* showing an area from which sectioned material was obtained. *B*. Fruiting body, submerged in the agar, producing only scolecospores A mass of these spores had been discharged into the agar at a lower level, only a small part of which (indicated by arrow) is visible in this section. (× 140.) *C*. Scolecospores and 1-septate pycnospores (not stained). The dark-colored pycnospores are out of focus because of their greater thickness. (× 350.) *D*. Fruiting bodies submerged in the agar. Their walls are united for a short distance. Each body produced and is discharging one type of spore, though at different levels; *p*, pycnospores; *s*, scolecospores. (× 148).

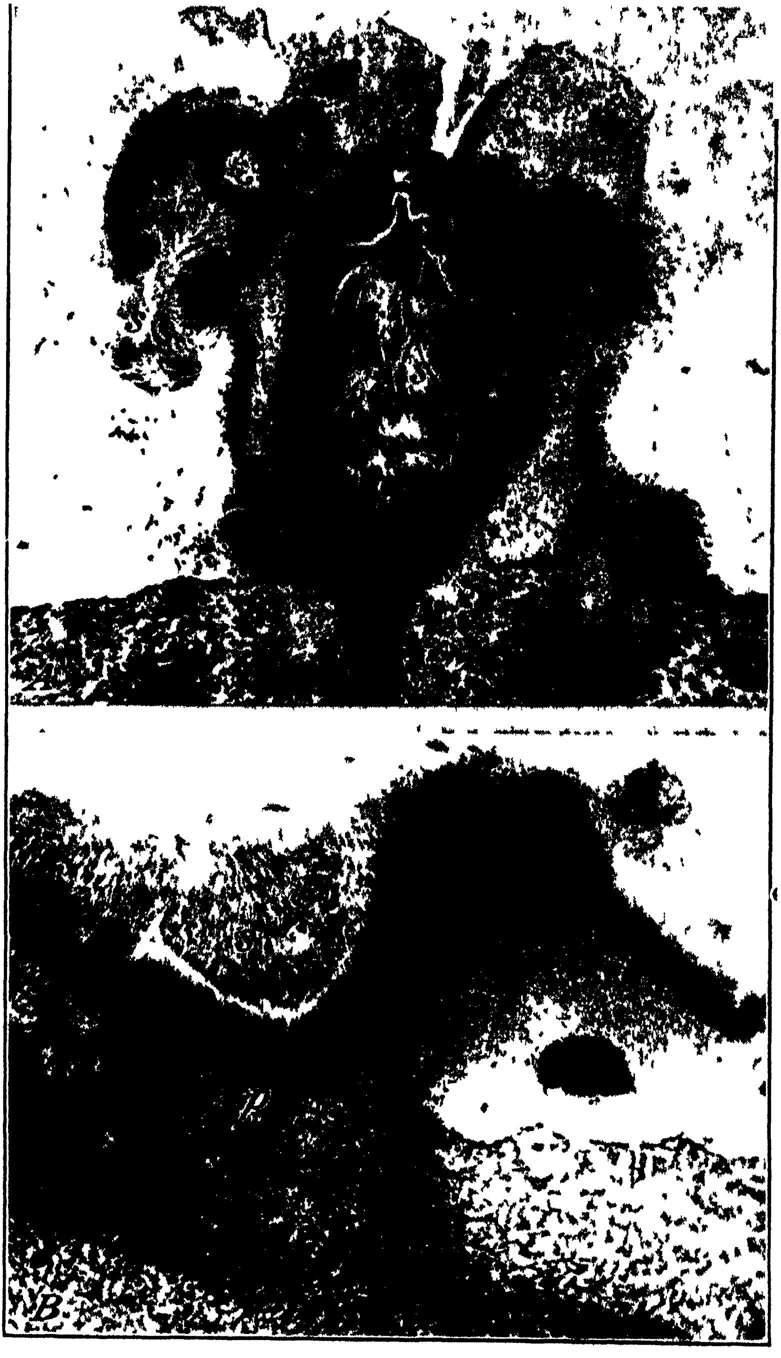

FIG. 2. Fruiting bodies, 41-day old culture, producing scolecospores on the surface of the plate. *p*, pycnospores; *s*, scolecospores. (*A*, × 46; *B*, × 55.)

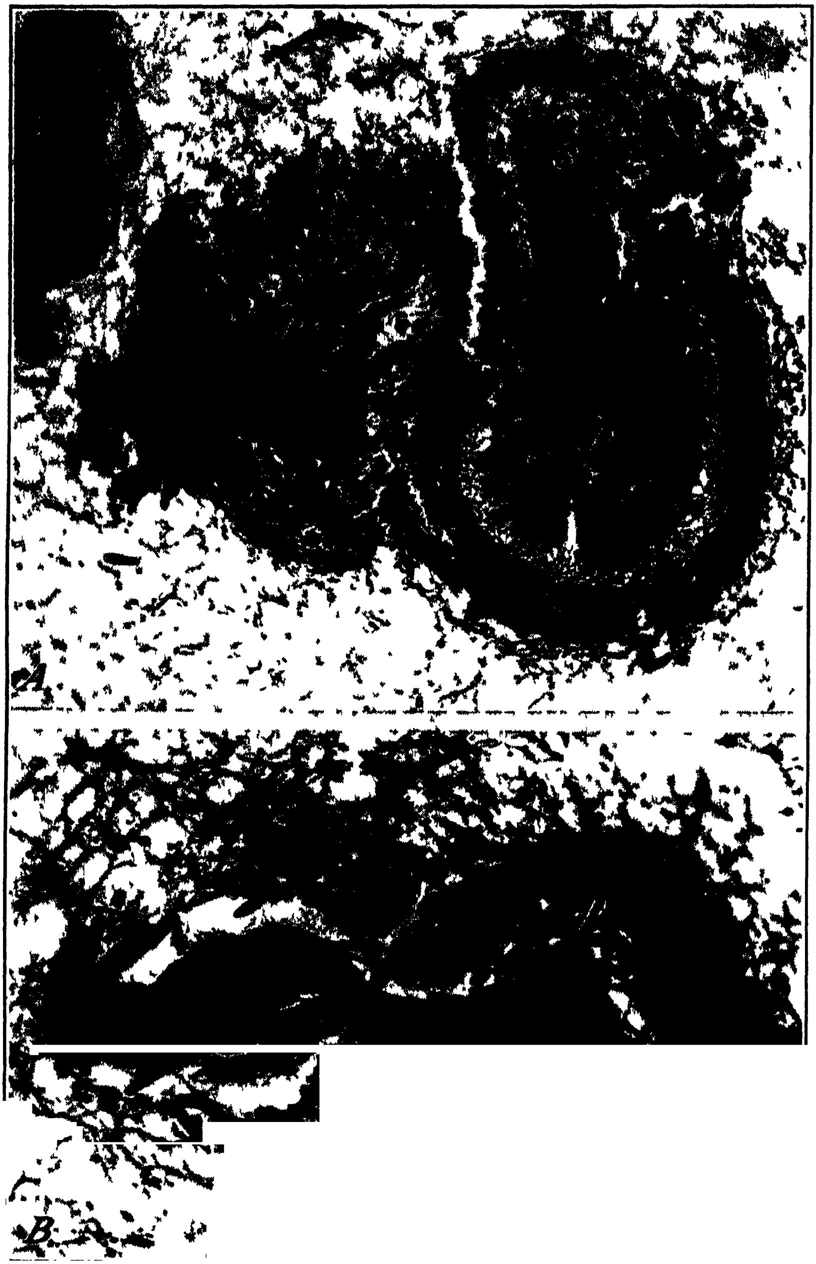

FIG. 3. Fruiting bodies, 42-day-old culture, submerged in the agar, containing both scolecospores (*s* and *s′*) and 1-septate pycnospores (*p*), the two types of spores more or less segregated Examination of the serial sections showed that in *A* the 1-septate spores predominated and in *B* the scolecospores predominated. *A*, *s′*, part of the mass of scolecospores discharged from the fruiting body shown in fig. 1, *B*. (*A*, ×180; *B*, ×250.)

Little is known concerning conditions that favor the production of these spores; seldom have they been found in a pure culture of a single colony. The presence of a different strain of *Diplodia zeae* appears to stimulate their formation. So far, the best method of obtaining scolecospores seems to be to plant an averting strain in the same plate or flask with the producing strain and allow the cultures to grow in a dimly lighted corner of the laboratory. Seemingly also, scolecospores are formed in greater abundance in flasks that are tightly capped than in those plugged with cotton. To obtain an abundance of pycnospores, on the other hand, it has been our practice to use plugged flasks placed in strong diffuse light.

Attempts to germinate the scolecospores in hanging drops of liquid or agar have not been successful.

The scolecospores are hyaline, threadlike, ranging in length from 21 to 45 μ, the majority being between 25 μ and 35 μ long (Fig. 1, *C*). They are born in fruiting bodies very similar in structure to the usual pycnidia entirely submerged in the agar (Fig. 1, *B* and *D*), or in simple to lobulate and cap-shape forms supported in pseudoparenchymata on the surface of the plate (Fig. 2, *A* and *B*). Fruiting bodies containing brown bicellular pycnospores and those producing scolecospores occur separately or in close contact, and, in some cases, the 2 types of spores, more or less segregated, have been found in a single pycnidium (Fig. 3).

No information is at hand concerning the distribution of such strains of *Diplodia* in the Corn Belt, nor is it known whether it is possible to induce this type of fruiting generally in culture.

Butler[3] has described a fungus, *Hendersonina sacchari,* pathogenic on sugar cane in India, which produces *Diplodia*-like pycnospores, and scolecospores that, in appearance, closely resemble those of the corn fungus, although they differ somewhat in spore measurements. Though these forms appear to be closely related, the question of nomenclature is being left for future consideration.

Photomicrographs of the fruiting bodies containing scolecospores are here presented to show a type of spore production hitherto unreported in *Diplodia zeae* and to suggest the possibility of the occurrence of similar structures in other strains of this fungus from the Corn Belt.

[3] Butler, E. J., and Abdul Hafiz Khan. Some new sugar cane diseases. India Dept. Agr. Mem. Bot. Ser. 6: 191–203. 1913.

GERMINATION EXPERIMENTS WITH OVERWINTERED TELIOSPORES OF TRANZSCHELIA PRUNI-SPINOSAE[1]

JOHN C. DUNEGAN

(Accepted for publication August 23, 1938)

INTRODUCTION

The inoculation experiments of Tranzschel (6) in Europe in 1904 and Arthur (1, 2) in United States in 1905-06 proved that the rust fungus, *Tranzschelia pruni-spinosae* (Pers.) Diet., is heteroecious. A review of the literature, in connection with the demonstration by the writer (4) that there are 2 varieties of this fungus, failed to reveal any record of the germination of the teliospores.

While this lack of knowledge concerning the germination of the teliospores does not affect the well-established fact that the rust is heteroecious, the successful germination of the teliospores would pave the way for the inoculation of ranunculaceous hosts with basidiospores, and thus aid in further clarifying the host relations of the *typica* and *discolor* varieties.

Furthermore, as pointed out by the writer (4), the teliospores of the *typica* variety consist of 2 cells, approximately equal in size and of similar shape and color. The variety *discolor,* on the other hand, is characterized by teliospores having the 2 cells of unequal size, of different shape, color, and wall thickness. Since these marked morphological differences exist between the teliospores of the 2 varieties, it is a matter of considerable interest to ascertain whether or not differences also exist in their mode of germination. The distorted shape and small size of the basal cell in many collections of the *discolor* variety suggest the possibility that only the apical cell of the teliospore is functional in this variety.

In the following pages the writer will discuss various germination experiments performed from 1924 to 1938 and will describe the promycelia, basidia, and basidiospores produced by the *typica* variety teliospores. The teliospores of the *discolor* variety did not germinate in these experiments.

MATERIALS AND METHODS

Three methods were used in overwintering the teliospore material; (a) the leaves were hung outdoors in cheesecloth bags; (b) the leaves, after being collected from the tree, were placed on the ground in an orchard; and (c) leaves were collected from under the trees, where they had overwintered *in situ.*

In the germination experiments the teliospores were removed from the sori and either floated in hanging drops of tap water or scattered over the surface of a thin layer of water agar[2] in Petri dishes.

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[1] Cooperative investigations between the Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Department of Plant Pathology, Arkansas Agricultural Experiment Station.

[2] Agar 15 g.; distilled water 1 l.

TABLE 1.—*Summary of teliospore germination experiments, 1924–1937*

Date leaves were collected	Variety of *Tranzschelia pruni-spinosae*	Host	Source of material	Treatment of material	Germination tests	
					Date	Results
Nov. 21, 1923	*typica*	*Prunus angustifolia* Marsh.	Georgia	Leaves placed outdoors in cloth bags	Feb. 16, 1924	Negative
Nov. 7, 1924	do	*P. serotina* Ehrh.	do	do	Feb. 24, 1925	Positive—but no basidiospores observed
do	do	do	South Caroli	do	do	Negative
Nov. 21, 1925	do	do	do	do	Jan. 22, 1926	do
do	do	do	do	do	Feb. 26, 1926	do
do	do	do	Georgia	do	do	do
do	do	do	South Carolina	do	Apr. 10, 1926	do
Jan. 31, 1930	do	*P. angustifolia*	Arkansas	Leaves collected from ground under trees	Jan. 31, 1930	do
Jan. 17, 1934	do	do	do	do	Jan. 18, 1934	do
Nov. 10, 1934	do	do	do	do	Dec. 11, 1934	do
do	*discolor*	*P. davidiana* Franch.	Georgia	Leaves placed outdoors in orchard	do	do
do	do	do	do	do	Jan. 15, 1935	do
do	do	*P. persica* S. & Z. var. *potanini*	do	do	do	do
do	do	*P. mexicana* Wats.	do	do	do	do
do	do	*P. besseyi* Bailey	do	do	do	do
Nov. 12, 1934	*typica*	*P. angustifolia*	Arkansas	Leaves collected from ground under trees	do	do
Nov. 10, 1934	*discolor*	*P. davidiana*	Georgia	Leaves placed outdoors in orchard	Feb. 27, 1935	do
do	do	*P. persica* var. *potanini*	do	do	do	do
do	do	*P. mexicana*	do	do	do	do
do	do	*P. besseyi*	do	do	do	do
March 2, 1936	*typica*	*P. angustifolia*	Arkansas	Leaves collected from ground under trees	Mar. 3, 1936	do
Feb. 2, 1937	do	*P. serotina*	do	do	Feb. 3, 1937	do
Feb. 27, 1937	do	do	do	do	Feb. 28, 1937	do
March 23, 1937	do	do	do	do	Mar. 24, 1937	do

All hanging-drop cells and Petri-dish cultures were examined within 24 hours. Occasionally, the observations were continued an additional 24 hours, but the cultures generally were so overrun with contaminations by this time that only the observations made during the first 24 hours were considered in the final results.

GERMINATION EXPERIMENTS FROM 1924 TO 1937

In 1927 Maneval (5) reported that teliospores from leaves of the peach, *Prunus persica* S. & Z., failed to germinate in November and teliospores from wild cherry, *P. serotina* Ehrh., failed to germinate in October. In both cases the spores were of the current season and the tests had been made soon after the spores were formed.

During the period 1924 to 1937 the writer attempted, at various times during the late winter months, to germinate the overwintered teliospores of the *typica* and *discolor* varieties of *Tranzschelia pruni-spinosae.* These experiments, summarized in table 1, gave negative results, except the test made on Feb. 24, 1925. In this test 10 teliospores of the *typica* variety developed promycelia, but no basidiospores were observed attached to, or in the vicinity of, the sterigmata.

GERMINATION EXPERIMENTS IN 1938

In the 1938 experiments, leaves bearing overwintered teliospores were collected from under wild-cherry trees in the vicinity of Fayetteville, Arkansas. Since the leaves were collected from the spot where they had overwintered *in situ,* exact data are lacking concerning the length of the overwintering period, but they had been on the ground at least 83 days when the first germination tests were started on February 11, 1938.

The various experiments carried out during the period February 11 to May 24 are summarized in table 2, and, in contrast to the results obtained previously, the teliospores of the *typica* variety germinated readily in 1938.

In the initial stages of teliospore germination a smooth-wall, hyaline promycelium, resembling an ordinary germ tube, emerges through a pore in the wall of either the apical or basal cell of the teliospore. Only one promycelium is produced from each cell, but both cells frequently germinate. As the promycelium elongates, the dense mass of cytoplasm, which completely fills the very young promycelium, is visible only in the apical portion, leaving a basal region of varying length almost devoid of cytoplasm. When the teliospores are germinated in hanging-drop cells, this basal portion frequently is 300 μ long, but, when the spores are germinated on the surface of water agar, the sterile basal portion of the promycelium is much shorter, rarely being more than 80 μ long.

The formation of the basidium is initiated by the development of 4 cross walls in the apical portion of the promycelium dividing the dense mass of cytoplasm into 4 cells, each containing a single nucleus and definitely separating the basidium from the basal portion.

TABLE 2.—*Summary of teliospore germination experiments performed in the spring of 1938*

Date	Host	Treatment	Observations		
			Date	Results	Remarks
Feb. 11, 1938	*Prunus serotina*	Teliospores placed in hanging drop cells (tap water).	Feb. 10, 1938	Promycelia, basidia and spores produced	Spores started to germinate after 5 hours.
Feb. 14	do	do	Feb. 15	Only promycelia formed —no spores.	
Feb. 15	do	do	Feb. 16	Very profuse, germination—many basidio spores.	Germination started after 5½ hours.
Feb. 17	do	Teliospores placed in hanging-drop cells of tap water and also scattered over surface of water agar plates.	Feb. 17	do	Promycelia developed above surface of agar.
Feb. 24	do	do	Feb. 25	No germination in hanging drop cells but teliospores on water agar germinated profusely.	Spores on leaves collected Feb. 10 and 14 and kept dry were still viable.
March 2	do	Teliospores scattered over surface of water agar plates.	March 3	Profuse germination — many basidiospores produced.	Teliospores were from leaves collected February 24 and kept dry.
March 24	do	do	March 25	do	Teliospores were from leaves that had been kept dry for 10 days.
April 4	do	do	April 5	do	Teliospores were from leaves collected Feb. 10, 17, March 14 and 23, and allowed to dry out.
April 6	do	do	April 7	do	Teliospores were from leaves collected April 6.
May 16	do	do	May 17	Negative	Teliospores from leaves collected Feb. 14 and 23 and kept dry in laboratory.
May 24	do	do	May 25	Negative	Teliospores from leaves collected Feb. 10, 17, March 14 and April 6, 1938, and kept dry in laboratory.

The sterigmata develop as projections from the walls of the 4 basidial cells and the cycle of development is completed by the formation of the basidiospores that appeared first as small globose swellings developing on the tip of each sterigma. The cytoplasm and nucleus pass from each cell of the basidium through the sterigma into the developing basidiospores, leaving the cells of the basidium almost devoid of cytoplasm. A typical promycelium with 3 basidiospores attached to the sterigmata is illustrated in figure 1.

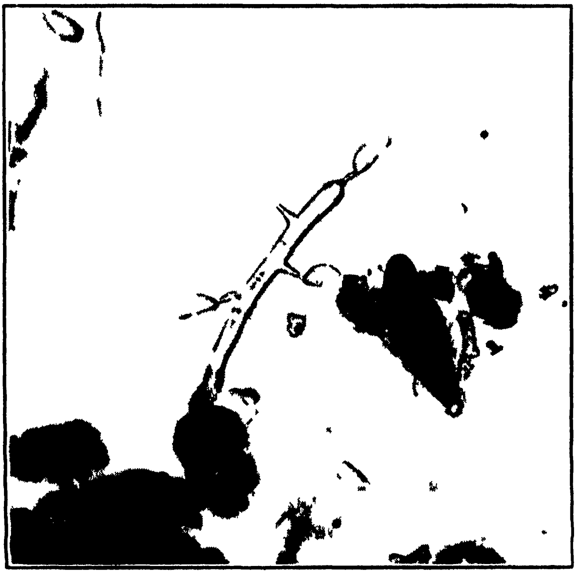

FIG. 1. Promycelium produced by overwintered teliospore of *Tranzschelia pruni-spinosae typica.* × 360.

The mature basidiospores are from 12.5 μ to 16 μ long and 5.5 μ to 6.5 μ wide with hyaline, smooth walls. When viewed from the side they are somewhat reniform, having one wall slightly curved and the other wall markedly so, giving the spore a pronounced bulge on one side. When viewed from above, the spore appears sub-elliptical with a rounded apex and slightly pointed distal end. The spore is attached to the sterigma near to, but not exactly at, the distal end of the side showing the greater curvature. A thin spot in the wall of the basidiospore marks the point of attachment to the sterigma and apparently also serves as a pore, for the germ tube emerges from the basidiospore at this point when the spores germinate.

DISCUSSION

It is not known whether or not the environmental conditions during the fall of 1937 and early part of 1938 were particularly favorable to the overwintering of the teliospores, but the overwintered teliospores of the *typica* variety germinated readily during the spring of 1938 after weathering outdoors for periods varying from 83 to 137 days. The teliospores that germinated in 1924 did so after a period of 109 days, but other teliospores (Table 1, experiments of April 10, 1926, Feb. 27, 1935, March 3, 1936, Feb. 28 and March 24, 1937) failed to germinate after being exposed outdoors for equally long periods.

On comparing the results obtained with hanging-drop cells and water agar, it was evident that much better results were obtained by the latter method, both in respect to the number of teliospores that germinated and the number of fertile basidia produced.

The teliospores of *Tranzschelia pruni-spinosae typica* are not unique in the production of long promycelia from spores in hanging-drop cells. Blackman (3) reported that the teliospores of *Puccinia graminis* Pers., *Uromyces fabae* (Pers.) de Bary, and *Phragmidium rubi-idaei* (DC.) Karst, immersed in water produced long promycelia, but the basidiospores were not produced until the promycelia grew into the air. Similarly, Weimer (7) found that the teliospores of *Gymnosporangium juniperi-virginianae* Schw. produced long promycelia, if the spores were immersed in water.

Occasionally, other abnormalities were noted among the spores of *Tranzschelia pruni-spinosae typica* germinated in hanging drops in which, in addition to an abnormally long promycelium, the sterigmata, instead of being short projections surmounted by basidiospores, appeared as hyaline thread-like branches from 40 μ to 60 μ long.

The basidiospores apparently are dislodged from the sterigmata with considerable force. In one instance 25 basidiospores were counted about a mass of promycelia and the individual spores were scattered over the surface of the agar from 200 μ to 630 μ from the center of the mass, the majority lying between 210 μ and 330 μ from the promycelia. In another case 13 spores were counted about a group of promycelia and the spores were from 100 μ to 500 μ from the promycelia.

The fact that all of the teliospores did not germinate in any one test, and that further tests with spores from the same collections showed additional viable teliospores indicates that the teliospores in any one collection probably reach maturity at different times. Furthermore, teliospores germinated, when scattered over the surface of water agar, after they had been kept dry in the laboratory for periods from 6 to 53 days. These results suggest that under natural conditions basidiospores are produced outdoors at irregular intervals, *i.e.*, after each rain that saturates the spores, for a period of several months in the spring.

The morphological differences between the teliospores of the *typica* and *discolor* varieties were mentioned earlier in this paper when the question was raised whether or not the basal cell of the *discolor* variety of teliospore is functional. For the present this question cannot be answered, since the results reported in this paper deal entirely with the germination of the *typica* variety of teliospores. The results obtained will, however, furnish a basis for comparison with the behavior of the *discolor* variety of teliospores in future tests.

SUMMARY

Teliospores of the *typica* variety of *Tranzschelia pruni-spinosae* overwintered on fallen leaves of *Prunus serotina* germinated when tested at various times from February 11 to April 6, 1938.

Teliospores scattered over the surface of water agar in Petri dishes germinated profusely with the production of short promycelia and the formation of basidia and spores.

Teliospores in hanging-drop cells of tap water germinated less profusely, the promycelia, basidia, and sterigmata frequently were abnormal in structure, and spore formation was suppressed at times.

The morphological characters of the promycelia, basidia, and basidiospores are described and it was found that both cells of the *typica* variety of teliospore are able to produce these reproductive structures.

The results obtained in the various germination experiments suggest that basidiospore production, under natural conditions outdoors, occurs at irregular intervals, depending upon the frequency of rainy weather for several months in the spring.

Teliospores of the *discolor* variety did not germinate in any of the experiments. The results obtained with the *typica* variety of teliospores, however, furnish a basis for comparison with the behavior of the *discolor* variety of teliospores in future tests.

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DOWNY MILDEW INFECTION OF FLUE-CURED TOBACCO IN THE FIELD[1]

J. A. PINCKARD AND LUTHER SHAW

(Accepted for publication Aug. 29, 1938)

Infection of tobacco, in the field, with *Peronospora tabacina* Adam was widespread in the Old Belt of North Carolina and Virginia in 1938. While the disease has been reported to cause serious damage to field-grown tobacco in Australia,[2] and while it has been noted as being present on various types of tobacco in the United States[3] since 1921, no accurate description of the field symptoms and of the conditions under which they developed have been

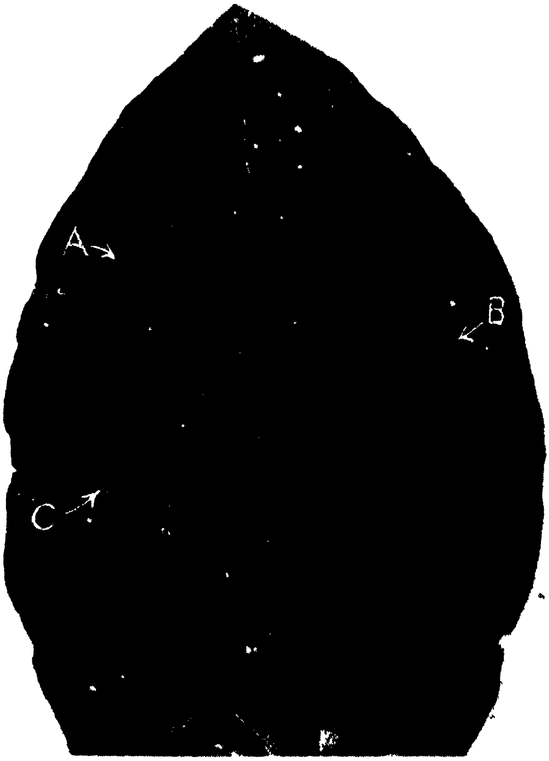

FIG. 1. Symptoms of downy mildew on old seed bed leaf of Yellow Mammoth tobacco, June 11, 1938. A. Group of small necrotic lesions. B. Lesions coalesced to form large indefinite leaf spot. C. Large lesion originating from chlorotic area, similar to those illustrated in figure 2. × $\frac{2}{3}$.

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[1] Cooperative investigations from the Virginia Agricultural Experiment Station, Tobacco Research Laboratory, Chatham, Va., and the North Carolina Agricultural Experiment Station, Extension Service, Raleigh, N. C.

[2] Angell, H. R., and A. V. Hill. Downy mildew (blue mould) of tobacco in Australia. Austral. Adv. Counc. Sci. and Indust. Res. Bull. 65: 1–30. 1932.

[3] Pinckard, J. A., and Luther Shaw. Downy mildew infection of tobacco plants in the field. U. S. D. A. Plant Dis. Rep. 22: 202–203. 1938.

made. The purpose of this report is to describe the symptoms of the disease on flue-cured tobacco, as they appeared in the Old Belt of North Carolina and Virginia in 1938, and to describe the conditions under which the epidemic developed.

The symptoms of downy mildew on flue-cured tobacco in the field were observed to follow two rather distinct courses. First, there appeared necrotic spots, usually grouped, giving a blotched effect (Fig. 1, A). These blotched areas appeared to represent a more or less static condition, since many were observed that persisted without material change until early July. Sporulation was not observed on this type of lesion, although it is probable

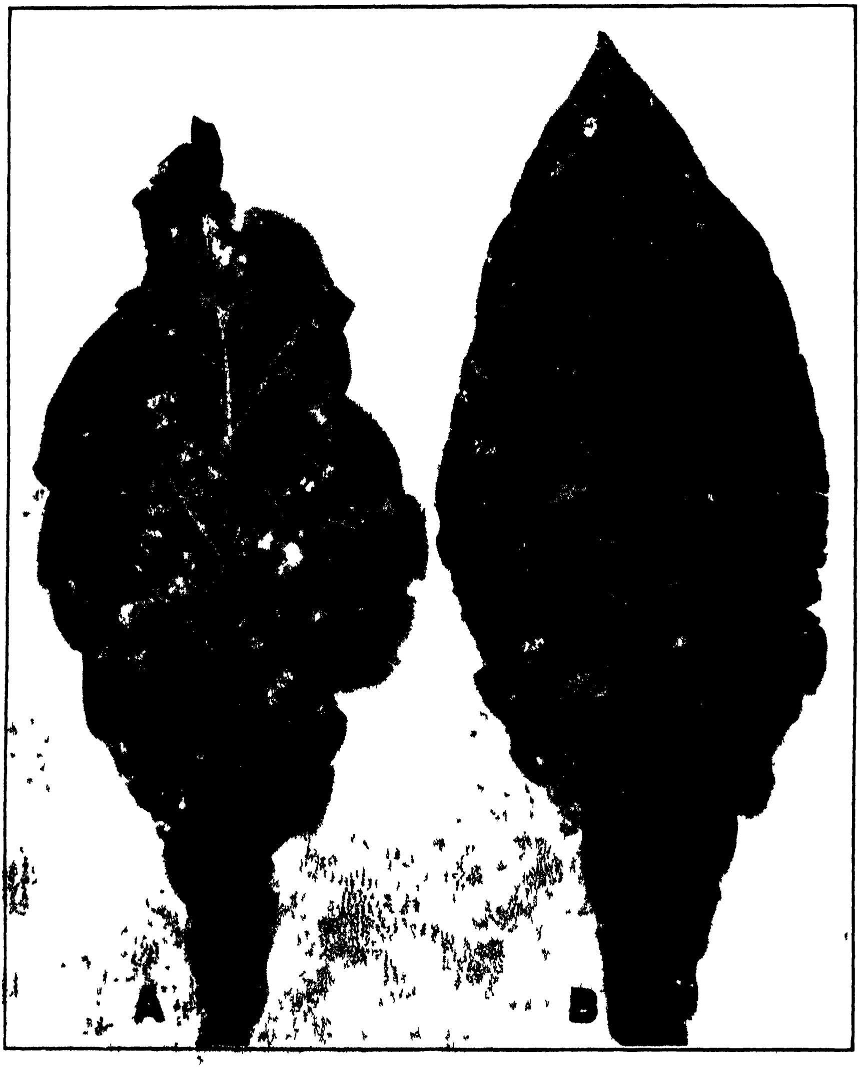

FIG. 2. Chlorosis and distortion of leaf of Yellow Mammoth variety of tobacco infected with downy mildew collected in the field June 11, 1938.

that sporangia were formed sparsely when temperature and moisture conditions were suitable. The individual small lesions bore considerable resemblance to those produced by *Bacterium angulatum*. However, the geometrical pattern of the blotched areas conformed so closely to those commonly observed on older leaves in the seed beds, Fig. 1, that there was little doubt as to their identity. Certain of the blotched areas were observed in which the small necrotic lesions coalesced forming large necrotic areas usually 10 to 20 mm. in diameter (Fig. 1, B). The margins of these lesions were usually somewhat indefinite and were unattended by marked chlorosis.

Second, there appeared chlorotic areas, varying widely in size and numbers per leaf (Fig. 2). Their margins were indefinite except where bounded by the larger veins. The leaves were frequently puckered (Fig. 2, A). These chlorotic lesions eventually developed into large, well defined, necrotic lesions (Fig. 1, C). Sporulation, while not abundant, was observed on these lesions (Fig. 3), and was most marked after the tissues had begun to necrose.

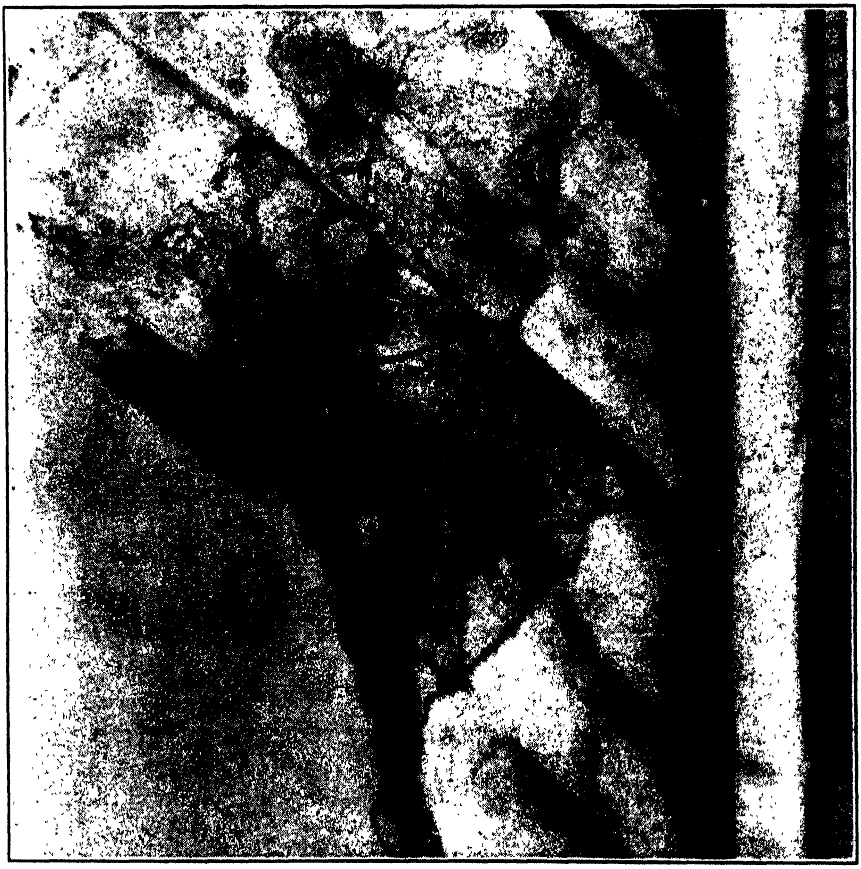

FIG. 3. *Peronospora tabacina* fruiting on lower surface of lesion of field-grown tobacco leaf collected June 11, 1938. x 2.

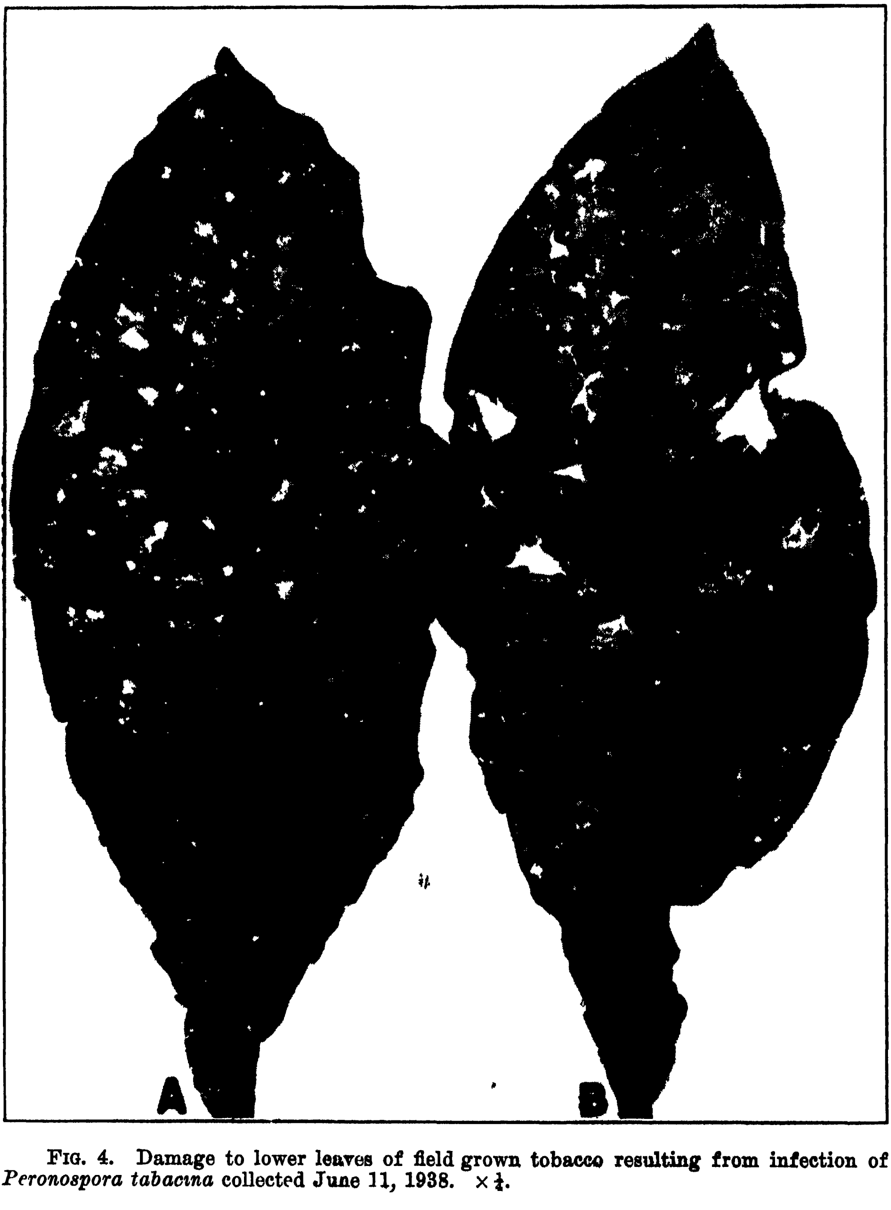

FIG. 4. Damage to lower leaves of field grown tobacco resulting from infection of *Peronospora tabacina* collected June 11, 1938. × ¼.

The lesions frequently coalesced and involved most of the leaf area. Later they disintegrated and fell from the leaf, leaving ragged holes (Fig. 4).

Epidemiological factors of significance occurring during the critical period of disease development were as follows: During the period from April 24, through May 12, precipitation was very light in the Old Belt. Of 700 daily weather readings from 35 stations[4] in the 20-day period, only 21 readings showed precipitation in excess of 0.05 in. In this period, nearly all of the Old Belt crop of tobacco was transplanted. Much of it was placed in the field without having been attacked by the downy-mildew parasite, although the disease was present in a limited number of beds in the area. Beginning on May 13 and extending to June 1, 35 stations reported precipitation on 391 days during a total of 665. Total precipitation reported by the various stations for May varied from 2.1 to 10.3 in., most of which occurred during the latter half of the month. General precipitation throughout the month of June was well distributed with totals for the different stations ranging from 3.2 to 14.2 in.

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[4] The weather records reported were taken from the climatological data of the U. S. D. A. Weather Bureau, North Carolina Section, for the periods indicated.

The mean temperatures for the Old Belt area were 60.4° F. for April, which was 1.4° F. above normal; 69.1° F. for May, which was 1.0° F. above normal; and 73.3° F. for June, which was 2.2° F. below normal. Beginning May 13, and extending through June, there was an excessive amount of cloudy weather.

Downy mildew symptoms first appeared on tobacco in the field in the Old Belt about June 1. The disease continued to spread over the area during most of June, although most rapid spread occurred during early June. A hot dry period beginning June 30, and extending into July, appeared to check development of the disease. Leaves well up on the stalks were involved, but damage of consequence was noted only on the lower leaves (Fig. 4).

Serious disease development in tobacco seed beds was observed in the latter part of May prior to the initiation of downy mildew in the field. Since viable sporangia were frequently collected from farm seed beds, inoculum for infection in the field probably came from these centers. The rainy period in late May evidently provided favorable conditions for dissemination, infection, and field development of the disease. From these observations, therefore, it would seem desirable to offer the suggestion that seed beds be destroyed as early as possible after the crop has been transplanted to the field.

ULTRACENTRIFUGATION OF JUICES FROM PLANTS AFFECTED BY TOBACCO NECROSIS

W. C. PRICE AND RALPH W. G. WYCKOFF

(Accepted for publication Sept. 1, 1938)

Characteristic macromolecular[1] substances have been isolated from plants affected by tobacco mosaic (16, 17) and certain other virus diseases (19, 7, 4, 5). Since it has been shown repeatedly that infectiousness is intimately associated with these substances, a detailed study of their properties is a matter of great importance. Some of the substances, such as those associated with the various strains of tobacco-mosaic virus, including the possibly related cucumber viruses 3 and 4 of Ainsworth (18, 1, 3), the latent-mosaic virus of potato (7, 4), and the bushy-stunt virus of tomato (11, 5), have proved suitable for such investigation because they are all relatively stable and occur abundantly in diseased plants. The virus of tobacco necrosis, which is unrelated to that of tobacco mosaic, was first described by Smith and Bald (12) and is now known to be about as infectious and about as resistant to various physical and chemical treatments as the virus of tobacco mosaic. For these reasons, it seems likely to prove especially useful for physical and chemical studies on the nature of viruses. In initiating such studies, we have subjected juices of plants affected by tobacco necrosis to ultracentrifugation and have in this way obtained a relatively stable macromolecular substance with which is associated a high degree of infectiousness.

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[1] The term "macromolecular" is used to characterize a material that sediments in the analytical ultracentrifuge at a rate indicating that its particles are similar in weight to the molecules of substances like hemocyanin. Whether or not these particles really are molecules is at present unknown.

PROPERTIES OF THE VIRUS

For the benefit of those who are not familiar with the literature on the tobacco-necrosis disease, it seems well at this point to review briefly some of the properties of the virus and some characteristic symptoms of the disease. The virus produces necrotic lesions in inoculated leaves of plants in at least 10 different families (13) but does not become systemic in any known host. In this respect it is unique among all known plant viruses. From 18 to 26 hours after inoculation, Turkish tobacco, *Nicotiana tabacum* L., leaves develop small lesions that gradually enlarge until they reach a diameter of 1 to 2 mm. (10). The lesions are illustrated in figure 1. Isolated lesions develop secondary rings and thus appear zonate. When many of them are present they may cause collapse and death of the entire leaf. Similar necrotic symptoms are produced on most of the known host plants. The lesions may be small, as in *N. glutinosa* L., or angular, with a tendency to follow leaf veins, as in cowpea, *Vigna sinensis* Endl., and bean, *Phaseolus vulgaris* L. Although the virus does not become systemic, it is capable of infecting roots of susceptible plants when added to soil in which such plants are growing (14, 10). Smith (15) has reported it to be air-borne, but this has not yet been confirmed (10).

Tobacco-necrosis virus has a thermal inactivation point of from 90° to 92° C. (10) and remains viable in extracted juice for at least 20 days at room temperature (12). It is infectious after drying for several months (14), after exposure to 100° C. dry heat for 15 minutes (14), and after storage in absolute alcohol for 6 months (14). It has been demonstrated (10), by means of cross-protection tests, to be distinct from tobacco-mosaic, tobacco ring-spot No. 1, tobacco ring-spot No. 2, cucumber-mosaic, and severe-etch viruses.

METHODS

Plants that served as a source of virus for our studies were grown in 4- or 6-inch porous clay pots in a greenhouse that was fumigated at frequent intervals. After they had reached a height of 6 or more inches, the plants were inoculated by rubbing the leaves with a gauze pad saturated with infectious juice, the lower fully expanded leaves usually proving to be the most susceptible. Leaves bearing large numbers of necrotic lesions were harvested from 3 to 8 days after inoculation and ground either immediately or after a preliminary freezing. The juice was expressed from the ground pulp by passage through cheese cloth and then clarified by low-speed centrifugation followed by filtration through a mat of celite (Hyflo Standard-cel). Preliminary tests showed that the clarification process removed little if any of the virus activity (Table 1). The clear brown celite filtrate was successively fractionated in the quantity ultracentrifuge (2, 21, 22, 23). The

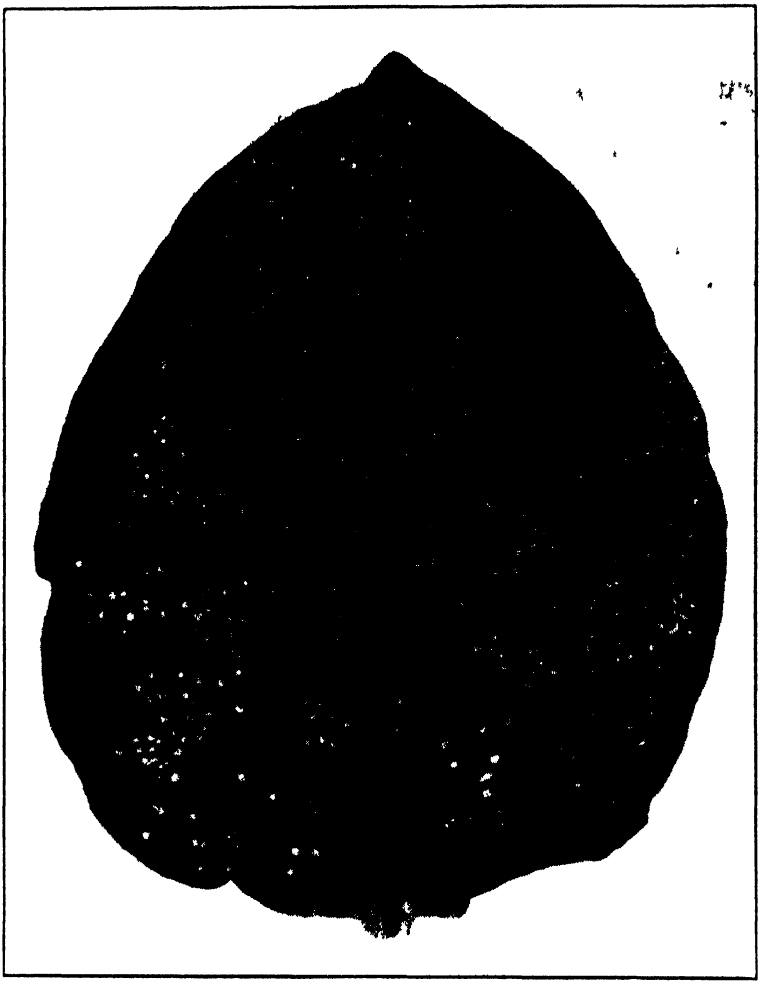


FIG. 1. Necrotic lesions produced in a leaf of Turkish tobacco by inoculation with tobacco necrosis virus. The photograph was taken 3 days after inoculation. (Photograph by J A. Carlile)

TABLE 1.—*Numbers of lesions produced in 28 cowpea leaves by inoculation with juice from diseased tobacco leaves before and after clarification by low-speed centrifugation and filtration through celite*

Logarithm of dilution	Original juice	Low-speed centrifugation	Celite filtrate
-2	389	480	610
-3	53	66	30
-4	2	7	3
-5	3	0	0

resulting fractions were examined in the analytical ultracentrifuge and the fractionation was adjusted to concentrate and purify the macromolecular substances that were found to be present. The various fractions, as well as the original starting material, were kept in an icebox at about 0° C. until they could be tested for infectivity. This was done by inoculating leaves of the Black variety of cowpea and counting the numbers of lesions produced.

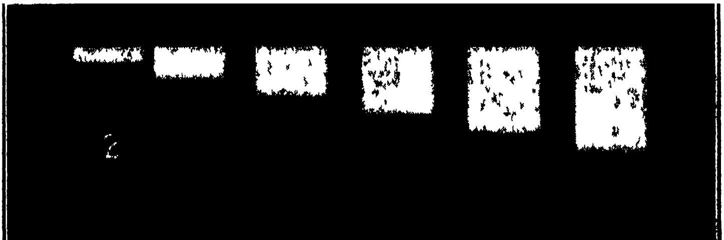
EXPERIMENTAL

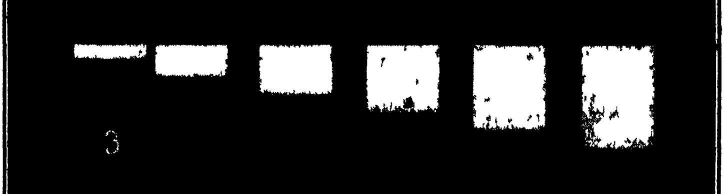
The Macromolecular Substance from Infected Turkish Tobacco Plants

Several different samples of juice from diseased Turkish tobacco plants were fractionated in the ultracentrifuge. In all cases the results were essentially the same. Most, though by no means all, of the infectiousness was concentrated in the pellet that formed in the centrifuge tube after one hour in a field of 60,000 times gravity. A solution of this pellet in saline of about $\frac{1}{4}$ the volume of the original juice was brownish and contained some flocculent material that was removed by low-speed centrifugation. At this stage sedimentable ultraviolet-light-absorbing substances were not, as a rule, present in sufficient quantity to be detected with the analytical ultracentrifuge. In one or two preparations, however, faint, sharp boundaries were obtained.

The process of ultracentrifugation followed by solution of the pellet and clarification of the resulting solution was repeated 3 or 4 times with each lot of diseased plant material. In every instance the final product was a highly infectious light brown solution of about a hundredth the volume of the starting material. It gave qualitative protein tests, no immediate Molisch reaction, and was apparently analogous to the virus proteins obtained from plants infected with other viruses. Its sedimentation pattern in the analytical ultracentrifuge was always the same—a single sharp boundary of the sort to be expected from a solution of an undamaged macromolecular substance (Fig. 2). The sedimentation constant calculated from photographs of each preparation has been the same. Its value, $s_{20°} = 112 \times 10^{-13}$ cm. sec.$^{-1}$ dynes^{-1}, is essentially identical with that of potato latent-mosaic (19, 7) and tobacco ring-spot (19) virus proteins.

The amount of macromoleculer substance producing these boundaries varied with the number and size of lesions on the leaves used. In one experiment, leaves harvested 5 days after inoculation yielded 760 cc. of sap after





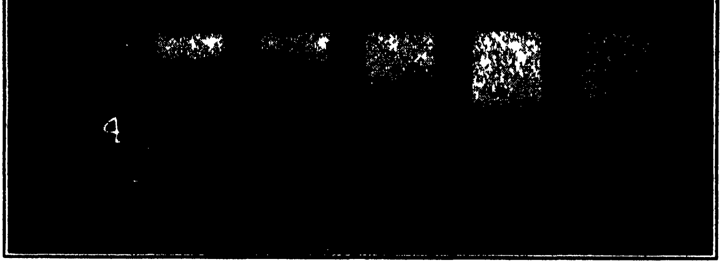


FIG. 2. Sedimentation diagram of a 3-times-ultracentrifuged suspension of the necrosis virus from Turkish tobacco plants. Interval between exposures = 5 minutes. Mean centrifugal field = 31,500 times gravity.

FIG. 3. Sedimentation diagram of a 4-times-ultracentrifuged suspension of the necrosis virus from cowpeas. Interval between exposures = 5 minutes. Mean centrifugal field = 32,000 times gravity.

FIG. 4. Sedimentation diagram of the twice purified sap from normal Turkish tobacco plants. Interval between exposures = 5 minutes. Mean centrifugal field = 41,300 times gravity.

freezing, grinding, pressing, and filtering through celite. The sap contained 1.05 mg. of nitrogen per cc. It was fractionated by means of 3 successive ultracentrifugations. The purified solution at the dilution used for ultracentrifugal analysis and for infectivity tests had a volume of 10 cc.; its nitrogen content was 0.16 mg. per cc. Because of losses during the ultracentrifugal fractionation, the content of macromolecular substance in the original infectious juice was undoubtedly somewhat greater than this indicates.

The infectiousness of the original plant juices, of the final purified product, and of partly purified solutions is summarized in table 2. Four separate tests with the celite filtrate from the original juice show the sort of agreement to be expected in such tests made at different times and serve for comparison with the final purified product. In this experiment the juice was clarified

TABLE 2.—*Numbers of lesions produced in leaves of cowpea with virus of tobacco necrosis in juice of Turkish tobacco plants before and after ultracentrifugation*

Logarithm of dilution[c]	Celite filtrate of original juice				Supernatant from low-speed centrifugation	Supernatant from one ultra-centrifugation		3 times ultracentrifuged			
	Test 1[a] 1-12-38	Test 2[b] 1-27-38	Test 3[b] 2-2-38	Test 4[b] 2-16-38	Test 1[a] 1-12-38	Test 1[a] 1-12-38	Test 4[b] 2-16-38	Test 1[a] 1-12-38	Test 2[b] 1-27-38	Test 3[b] 2-2-38	Test 4[b] 2-16-38
− 1	635			545	550	151	223				
− 2	252	383	419	422	248	27	42	386			
− 3	56	176	135	47	66	7	9	138	711	294	295
− 4	7	22	22	10	9	2	1	74	194	123	108
− 5	2	6	1	3	4		0	20	188	27	70
− 6		1	0					23	78	11	16
− 7		0	0					2	36	9	7
− 8								7	24	4	
− 9									7	4	
−10										2	
−11										0	
−12										0	

[a] Lesions in 28 cowpea leaves.
[b] Lesions in 32 cowpea leaves.
[c] Dilutions were made with saline.

by centrifuging for 15 minutes in a field of 5000 times gravity. The lesion counts obtained with the supernatant from this centrifugation indicate that very little virus was rejected with the sediment. The counts from the supernatant of the first ultracentrifugation when compared with those from the original juice give an idea of the amount of virus lost in this and subsequent ultracentrifugal sedimentations. It is calculated that this loss was roughly from 5 to 10 per cent of the virus in each sedimentation. The counts obtained with the 3-times-ultracentrifuged material give an indication of the amount of virus in this material. The titration end-point of the purified solution was about 10^{10}, that of the starting material about 10^{-6}. This result suggests that the purified solution was approximately 10,000 times more infectious than the original juice, although reduced in volume only 76 times. Somewhat similar results were secured with a different lot of infected material (Table 3). When the titration end-points are used for comparison, the 4-times-ultracentrifuged material of table 3 appears to be about 1000 times more infectious than the original juice, although reduced in volume only 50 times. The reality of this apparent enhancement of activity cannot be definitely established without more extensive investigations than we have been able to carry out. The implication that inhibiting substances may be present in sap from diseased plants and that such materials may be removed by ultracentrifugation suggests that further study of the matter would be worth while.

TABLE 3.—*Number of lesions produced in 32 cowpea leaves with tobacco-necrosis virus in juice of Turkish tobacco before and after 4 ultracentrifugations*

Logarithm of dilution[a]	Celite filtrate of original juice				4 times ultracentrifuged			
	Test 1 3–24–38	Test 2 3–29–38	Test 3 4–8–38	Test 4 4–27–38	Test 1 3–24–38	Test 2 3–29–38	Test 3 4–8–38	Test 4 4–27–38
1	667		724	590				
2	311	307	260	72	1161	1413	2014	
− 3	41	35	49	22	430	280	776	212
− 4	4	6	5	0	57	47	120	27
− 5	1	1	3	0	16	8	34	5
− 6	1	0	0		6	0	6	0
− 7		0	0		8	0	2	1
− 8					2	0	1	3
− 9					4	0	2	
− 10					8	1	2	

[a] Dilutions were made with saline.

The foregoing suggestions are based on the assumption that the dilution end-point of a virus sample is a legitimate index of its concentration. If, however, other portions of the dilution curve are used for comparison, somewhat different conclusions may be reached. When the data from tables 2 and 3 are plotted on logarithmic paper, the curves obtained are approximately straight lines. The calculated slope constants for these curves are given in table 4. The slopes for the ultracentrifuged material of table 2 are only about half those for the original juice. The data of table 3 do not show

TABLE 4.—*Calculated slope constants for the dilution curve data of tables 2 and 3 (Log Y = M log x + b)*

Virus sample	Test No.	Data of:			
		Table 2		Table 3[a]	
		M	b	M	b
Celite filtrate	1	0.656	3.588	0.646	3.516
	2	0.663	4.042	0.823	4.083
	3	0.865	4.553	0.648	3.572
	4	0.614	3.545	0.704	3.395
After low-speed centrifugation	1	0.571	3.415		
Supernatant from 1 ultracentrifugation	1	0.622	2.744		
	4	0.771	3.159		
After repeated ultracentrifugation	1	0.336	3.166	0.455	3.795
	2	0.306	3.705	0.752	4.675
	3	0.300	3.135	0.585	4.480
	4	0.408	3.719	0.813	4.737
Average for the 4 tests					
Celite filtrate		0.623	3.641	0.728	3.671
Ultracentrifuged		0.305	3.272	0.684	4.595

[a] In this section of the table some of the points in the more dilute range of the curve were definitely off the trend of the curve and were, therefore, not used in calculating slope constants.

so great a difference as is shown by those of table 2, but, even in this case, there is a considerable difference between the slopes for the purified material and the original juice. We are, at present, unable to explain these observations. They are, nevertheless, of importance in interpreting the activity measurements obtained. As previously pointed out, if the dilution endpoints for the virus samples are used for comparison, the purified material appears to be about 10,000 times more active than the starting material. This difference decreases, as other portions of the dilution curves are used for comparison, until at the more concentrated range of the curve the original juice, in some instances at least, appears to be somewhat more active than the ultracentrifuged material. This may be seen clearly by a comparison of the calculated b values in table 4 for the ultracentrifuged material and the celite filtrate of the original juice. From these data it is not possible to conclude whether or not the virus was concentrated by the ultracentrifugal procedure.

The Macromolecular Substance from Other Infected Host Plants

Macromolecular substances with essentially the same sedimentation constants were obtained from the sap of other host plants infected with tobacco-necrosis virus. Closely agreeing measurements on an excellent series of photographs of concentrates from diseased cowpea leaves gave an average value of $s_{20^\circ} = 112 \times 10^{-13}$ cm. sec.$^{-1}$ dynes^{-1}; the calculated constant for concentrates from diseased cucumber, *Cucumis sativus* L., leaves was $s_{20°} = 113 \times 10^{-13}$. The small amount of material in the end-fraction from juice of diseased *Nicotiana glutinosa* gave too faint and diffuse a boundary for accurate measurement, but a macromolecular component was evidently present. Accurate determinations of the yield of these substances could not be made because of losses during purification and the difficulty of freeing them from other materials, but the amounts obtained have been roughly proportional to the infectiousness of the starting material. The smallest yield was from *N. glutinosa*. It was less than 2 per cent of that obtained from Turkish tobacco. Diseased cucumber leaves were found to contain an intermediate amount of the macromolecular substance. Of the various host plants tested, however, the only source at all comparable with tobacco was the cowpea. Fairly pure infectious solutions of the substance were obtained by 5 ultracentrifugations of juice from this plant (Fig. 3). Results of infectivity tests with one lot of centrifuged and noncentrifuged juice from diseased cowpea leaves are summarized in table 5. Similar results were obtained with a second lot of material. As will be seen from the table, the filtered juice from diseased cowpeas was highly infectious when first obtained. It completely lost its activity, however, after storage for 10 days at 0° C. The reason for this loss has not been established, but it may well have been due to the injurious effect of bacteria that grew during storage. The macromolecular substance isolated from diseased cowpea leaves, when taken up in 1/50 the original sap volume, was (Table 5) about as infectious as the original juice but considerably less infectious than the similar material obtained from tobacco.

TABLE 5.—*Numbers of lesions produced in 32 leaves of cowpea with tobacco-necrosis virus from cowpea before and after ultracentrifugation*

Logarithm of dilution[a]	Celite filtrate					
	Tested immediately	Tested after storage at 0° C. for 10 days or more		Purified by 5 ultra-centrifugations		Supernatant from first ultracentrifugation
		Test 1	Test 2	Test 1	Test 2	
−0			0			100
−1	740	0	0			67
−2	212	0	0	183	200	10
−3	16	0	0	21	18	
−4		0	0	3	3	
−5		0	0	0	1	
−6		0	0	0		

[a] Dilutions were made with saline.

The Macromolecular Substances from Healthy Plants

Macromolecules from seemingly healthy tissues were first described in the course of studies on silkworms (6). They have been extracted from the juices of healthy broad bean, *Vicia faba* L., and pea, *Pisum sativum* L. var. *arvense* Poir., plants and from cucumber leaves (8, 9). In the experiments here reported such substances were encountered in high concentration not only in cucumber sap but in the juice of cowpeas. The large amounts of these substances and their comparative stability are responsible for the difficulties already mentioned in obtaining purified infectious virus solutions from these 2 hosts.

The previously described macromolecules from healthy broad-bean, pea, and cucumber plants have all sedimented with essentially the same constant —$s_{20^0} =$ ca. 77×10^{-13}. The healthy cucumber material available for the present study was not so fresh as that previously (9) used; its concentrate showed 2 boundaries, one with $s_{20^0} = 74 \times 10^{-13}$, the other with $s_{20^0} = 45 \times 10^{13}$. This second evidently represents a first stage in decomposition.

The macromolecules obtained from healthy cowpeas sedimented more rapidly than those from other plants. The measured constant was $s_{20^0} = 51 \times 10^{-13}$. The corresponding boundary in photographs of concentrates from diseased cowpeas yielded $s_{20^0} = 58 \times 10^{-13}$.

Although only one sedimenting boundary was observed in concentrates of diseased Turkish tobacco and *Nicotiana glutinosa* plants, we have made careful studies to see whether lighter macromolecules could be isolated from the juices of healthy plants of these species. It was found that such macromolecules may be extracted if distilled water is used for re-solution of the pellets obtained during the ultracentrifugal purification. From this work it seems likely that the failure to find such substances during the earlier ultracentrifugal studies with infected tobacco plants was probably due to the destructive action of the salt solutions then employed. In experiments with healthy Turkish tobacco leaves, the expressed juice was clarified by low-speed centrifugation and then ultracentrifuged for 1½ hours in a field of 60,000 times gravity. The resulting pellets were resuspended in ¼ the original sap volume of distilled water, clarified by low-speed centrifugation, and resedimented by a second ultracentrifugation. An aqueous solution of the pellets in a volume equal to a few tenths of a per cent of the original showed a sharp boundary in the analytical ultracentrifuge (Fig. 4). The sedimentation constant of the substance producing this boundary is $s_{20^0} = 74 \times 10^{-13}$. The same sedimentation constant was obtained after an additional ultracentrifugation. Tests made by rubbing the original juice and the purified macromolecular substance over the leaves of tobacco, cowpea, *N. glutinosa*, and cucumber plants failed to give any infection indicative of a virus contaminant. The macromolecules from healthy Turkish tobacco plants keep in the icebox for considerable periods of time. They are, however, easily destroyed by repeated ultracentrifugal sedimentations.

Some difficulty was experienced in detecting similar macromolecules in the sap of *Nicotiana glutinosa* plants. When, however, the entire experiment of grinding the leaves and expressing the juices, carrying through the ultracentrifugal fractionation at a low temperature, and making the necessary ultracentrifugal analysis was completed in a single day, a sharp boundary with $s_{20^0} = 76 \times 10^{-13}$ was obtained. The boundary, sedimenting at the same rate as the macromolecules from all the other healthy plants except cowpeas, was sometimes accompanied by a lighter boundary corresponding to $s_{20^\circ} = 38 \times 10^{-13}$.

DISCUSSION

Purified solutions of the macromolecular substances from Turkish tobacco plants having tobacco necrosis are relatively stable; when kept in an icebox, they maintain both their high infectivity and the sharpness of their sedimenting boundary over a period of at least 5 weeks. This high degree of stability conforms to the high degree of resistance to heat and other physical and chemical agencies exhibited by the virus of tobacco necrosis. In the present work it has not been possible to study the chemical composition of the macromolecular substance, but the evidence suggests that it is similar in nature to virus proteins isolated from other diseased plant tissues. On the assumption that it is a protein with the usual protein density and a normal diffusion constant, its molecular weight would be of the order of 9 millions.

The homogeneous macromolecular substances from various species of healthy plants may be significant not only for their possible relationship to the virus proteins, but also because of their possible functions in the physiology of living cells. From this point of view, it is noteworthy that all of these substances isolated from healthy plants have been pigmented. That obtained from cucumbers has a deep chlorophyll-green color; some of the others, such as the one from cowpea, are less strongly colored. It would seem to be of fundamental importance to determine how closely the macromolecules are associated with the chlorophyll in living cells. Macromolecular substances have been obtained from plants in widely separated families. It is a curious fact that all of these substances, except that of cowpea, sediment at the same rate. The explanation of this is not yet clear, but it is not incompatible with Svedberg's (20) suggestion that protein molecules of only a limited number of configurations are stable.

SUMMARY

A characteristic macromolecular substance has been isolated by quantity ultracentrifugation from the juice of Turkish tobacco plants infected with the virus of tobacco necrosis. Purified solutions of this substance sediment with a single sharp boundary and a constant of $s_{20^\circ} = 112 \times 10^{-13}$ cm. sec.$^{-1}$ dynes^{-1}.

Substances with the same sedimentation constants have been isolated from cucumber, cowpea, and *Nicotiana glutinosa* plants infected with the virus. The amounts of macromolecular substances obtained are roughly proportional to the infectiousness of the juices of the different host plants tested.

The infectious juices from diseased cucumber and cowpea plants contain lighter components that probably are the same as the pigmented macromolecular substances present in considerable amounts in healthy plants of the same species. Small amounts of similar non-infectious macromolecules have also been found in the juices of healthy Turkish tobacco and *Nicotiana glutinosa* plants. All these substances, except that of cowpea, have about the same sedimentation constant—$s_{20^\circ} =$ ca. 75×10^{-13} cm. sec.$^{-1}$ dynes^{-1}. That of cowpea sediments with $s_{20^\circ} = 51 \times 10^{-13}$.

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THE DEGENERATION OF METROPOLITAN BENT[1]

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INTRODUCTION

Diseases of turf have been recognized for many years. Old woodcuts depict players on very patchy greens, which would suggest a wide prevalence of disease even at an early date. The modern interest in turf diseases began in 1914, when F. W. Taylor described a disease of turf in Philadelphia, which was shown a year later to be caused by *Rhizoctonia solani* Kühn (9). When one considers the large financial interests directly involved, it is not surprising that turf diseases are attracting increasing attention.

Many diseases of turf are now known to be caused by fungi, but the cause of many others is as yet unknown; hence, their control is purely empirical and pragmatic (7). There is also a wide range in the types of grasses used on golf greens, each keeper having selected the strain that seemed best suited to the immediate environment, the majority of these strains arising as bud sports from the clonally propagated mother lawn. This diversity of strains leads to a lack of unity in cultural practices, which naturally increases the opportunity for the occurrence of disease.

The disease of Metropolitan bent which will be described has not been reported hitherto and, so far as the writer's experience goes, is limited to Minnesota and the neighboring States. However, it may not have been recognized in other regions or possibly may have been confused with the more common turf disorders.

SYMPTOMS AND GENERAL DESCRIPTION

The disease is confined to that variety of *Agrostis stolonifera* known as Metropolitan bent. On affected greens there is a general browning and death of the grass over large areas (Fig. 1). Typical brown patch (*Rhizoctonia solani*) is characterized by the localization of the infection to certain well-defined areas that may subsequently fuse, but in the case of the disease herein described, the death of the grass takes place generally, although it may be more rapid on different parts of the same green.

Leaf tips of affected plants have a greyish-brown discoloration, followed by a withering and progressive destruction of the whole leaf blade. The roots of affected plants are very short and brittle, breaking easily, even with the most careful handling. As the disease progresses, the green gradually becomes denuded until none or very little of the original grass is left.

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[1] The writer wishes to express his thanks to the many greenkeepers who assisted him during the course of the work, and to L. Feser of Wayzata, Minnesota, and A. Anderson of University Golf Course, St. Paul, Minnesota, in particular. Thanks are also due to Prof. E. C. Stakman for much helpful criticism throughout the work and for his kind assistance in the preparation of the manuscript.

[2] The work was done during the tenure of a Commonwealth Fund Fellowship at the University of Minnesota, in the Division of Plant Pathology.

Where the Metropolitan bent has died out, it usually is replaced by *Poa annua,* so that the whole green becomes mottled, with *Poa* growing upright, the remaining prostrate green bent, and the brown dead patches.

The survival of a few individual plants is a consistent and noteworthy feature. These are of a lighter green so that their appearance makes a spot of green here and there on the brown areas, giving rise to the name "freckle," by which the disease is known to the greenkeepers.

FIG. 1. Typical diseased area The dark scattered patches are *Poa annua* invading the dead turf, while the normal turf covering can be seen in the lower right hand corner.

ECOLOGY OF THE DISEASE

The time of appearance of the symptoms varies, pronounced symptoms having been seen both in spring and fall. However, they most commonly appear in spring or early summer, and in a short time (2–3 weeks) large areas of the green may become completely brown. The writer made his first observations in the late fall (November 1) and, even then, the destruction was well-nigh complete on diseased greens.

The question of both soil and air drainage was examined with respect to this degeneration, as it had been previously shown by Monteith (6) that pockets of still air increased the incidence of brown patch and that surrounding the green with trees decreased the air drainage. The degeneration under discussion, however, was just as prevalent and destructive in hollows as on exposed high ground or on slopes. Although the degeneration was very marked on some very well-sheltered greens, it was sometimes equally destructive on greens that were fully exposed to winds from all directions.

The type of subsoil on which this degeneration could be found varied from a light loam through soils rich in humus to heavy clays, indicating that the physical texture of the soil had little effect on either incidence or destructiveness. It would follow that the drainage or amount of moisture in the soil also could be said to be at least relatively unimportant.

One very constant factor on all the diseased greens examined was the extreme density of the turf mat. Metropolitan bent stolonates very freely, and a high percentage of these stolons take root and develop new plants, thus building up a dense and relatively impermeable surface. So dense is this mat that in many cases aeration is so greatly impaired that grass clippings and dead roots are not completely destroyed, and a top soil, in some cases 2 or 3 inches thick and composed of nothing but plant débris, is built up. This is probably caused by a lack of oxygen necessary to the bacterial destruction of the organic débris with the formation of humus and liberation of minerals and carbon dioxide.

ATTEMPTS TO ISOLATE A CAUSAL ORGANISM

Pieces of diseased roots, leaves, and stems were surface-sterilized in mercuric chloride or silver nitrate, washed in sterile distilled water, and plated on potato-dextrose agar and on Richard's agar. Other pieces were simply washed in sterile distilled water and isolations were made from the resulting fungal and bacterial colonies. Many organisms were thus obtained and used to inoculate healthy plants in the greenhouse.

The inoculum was increased on sterile oat hulls or potato-dextrose agar until a sufficient quantity of inoculum could be obtained for the purpose. Plants were inoculated by smearing the organism on the leaves immediately after clipping, by sprinkling a suspension of spores and mycelium on the plant and on the soil, and by inserting the fungus growing on oat hulls among the roots. Inoculations were made with 20 different isolates, but in no case was there evidence of pathogenicity.

A fine gray bloom of mycelium developed on diseased leaf tips incubated in a damp chamber; this fungus also was isolated and inoculated onto healthy plants, but proved to be non-pathogenic and was probably a saprophyte on the decayed leaves. None of the bacteria were pathogenic.

Examination of soil samples failed to reveal the presence of any grub or insect that might be the cause of the disease, and attempts to transmit the disease by rubbing healthy plants with diseased leaves failed also. This accumulation of negative evidence seemed to indicate that the degeneration was non-parasitic in origin.

CONTROL MEASURES

When the disease was first noted in the field, it was assumed to be similar to brown patch and snow mould in being caused by a fungus. Therefore, attempts were made to control it by means of chemicals, especially mercurials. Applications of mercurous and mercuric chloride did not control it. Similar experiments in the laboratory, using the mercury compounds and copper sulphate, also yielded negative results. The latter, when applied in too great concentrations, caused injury, as has been previously reported (1). These efforts to control the disease by chemicals furnish additional evidence that the disease is not caused by fungi, and, although this evidence is by no means conclusive, it is nevertheless cumulative.

Since the disease was not checked by chemicals it was thought that drainage and water availability might be of some importance. Tests were made in the greenhouse, as they also had been made by greenkeepers in the field, but no combination of watering schedules appreciably affected the severity of the browning. Excessive watering led to the appearance of a chlorosis along the edges and at the tips of the leaves, but the symptoms thus manifested had no resemblance to those of the disease under investigation.

To improve the aeration of the greens, they were very heavily spiked or opened up with an iron fork. The surface was then raked severely to break up the very dense surface mat and so allow the free circulation of air around the infected plants. A slight beneficial effect resulted, but it was neither permanent nor pronounced. Thus, although the grass grew better, the typical symptoms were still present and the improvement was only slight and temporary. This was true also of the application of fertilizers.

SUBSEQUENT INVESTIGATIONS

Various greenkeepers had noted that there was sometimes a recovery in the diseased areas, a new but rather poor turf being formed. This turf was of a slightly lighter green and probably resulted from the spread of the disease-escaping plants previously mentioned. In the pots in the greenhouse it was noted that, after 6 months, a number of the diseased turfs had recovered and were growing vigorously.

This recovery grass had, as has already been noted, a lighter green color

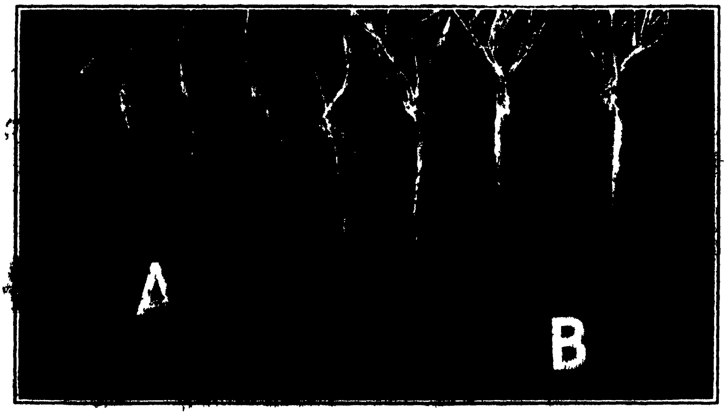

FIG. 2. The bunchy appearance of the escape grass and the much longer root system are apparent. A. Healthy Metropolitan bent. B. Escape grass.

than the original Metropolitan bent and a more bunchy appearance caused by more stems arising from the one point on the stolon (Fig. 2). There was also a very decided difference in the root systems of the two types, that on Metropolitan bent being only half as long as that on the new plants. In the light of the recent findings of O'Brien and Dennis (8), this difference in the length of the root system acquired a special significance and the investigations were extended.

A comparison was made between the root system of the Metropolitan bent, which was very subject to the trouble, and a variety known as Woodhill, which did not become affected. In plants from pots in the greenhouse, after 6 months' growth, the root system of Woodhill bent was more than twice as long as and many times more extensive than that of healthy Metropolitan bent grown under the same conditions. The average length of the 10 longest Woodhill roots was 17.7 cm., while for Metropolitan it was only 8.6 cm., and the difference in number, volume, and surface area can be seen in figure 3. Another very striking difference was the number of dead roots on the surface of the Metropolitan turf, which could easily be washed away, but, under natural conditions, would accumulate as a dense, relatively impermeable surface layer.

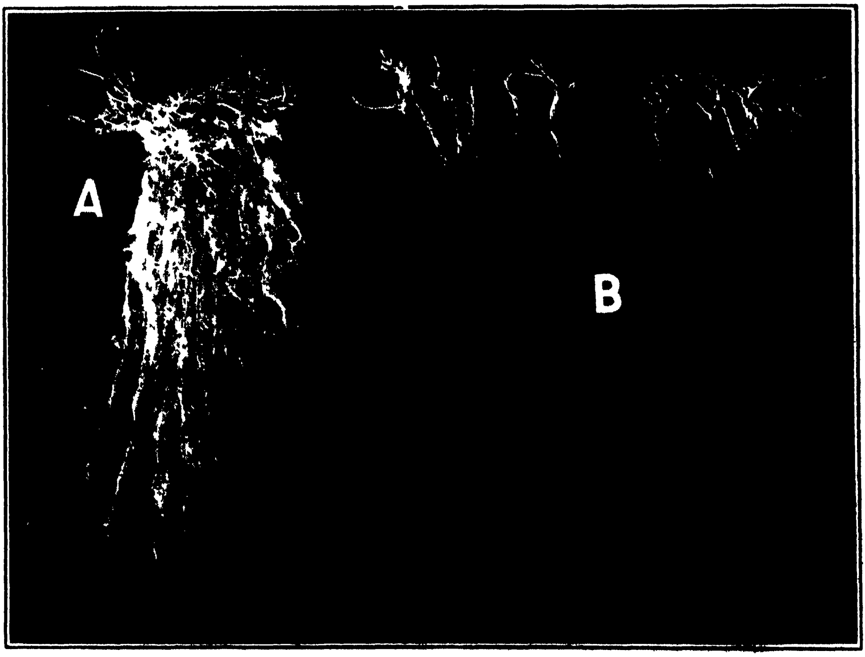

FIG. 3. These plants were grown in pots for six months before the photographs were taken, and exaggerate the normal difference in root development. A. Resistant Woodhill bent. B. Healthy Metropolitan bent.

Since the plants on which comparisons were made had been grown under very artificial conditions in the greenhouse, it was thought advisable to see if the differences prevailed in the field also. Pieces of turf 9 inches square and about 18 inches deep were taken from the greens and brought into the laboratory. They were then left to soak overnight and the soil washed off. When the whole turf had been treated thus and the roots could be separated easily, square pieces of equal size were taken from the centers of the original turfs and the root length examined. This latter selection was to obviate the chance of roots having been cut by the spade when the turf was dug out. The turf thus obtained can be seen in Fig. 4, where it is evident that the conditions seen in the greenhouse pots hold good for the field also. The roots of Woodhill were about twice as long and much greater in surface area than those of the Metropolitan bent. The difference in the respective root lengths thus seems to be consistent.

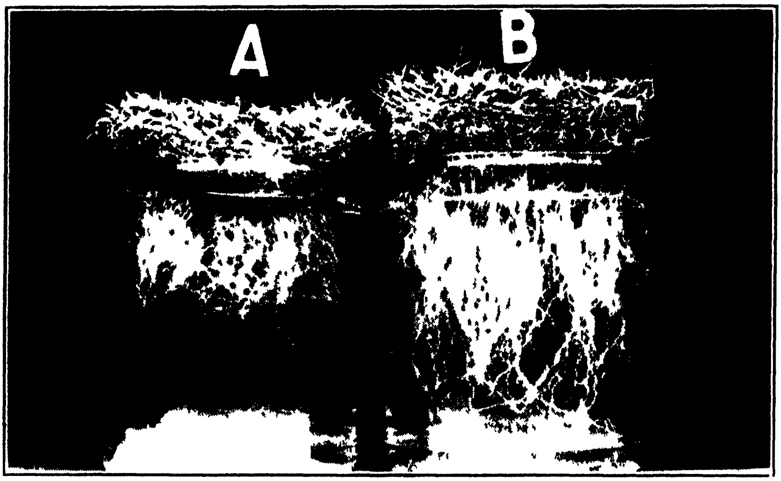

FIG. 4. Obtained from turf cut from the University Golf course, St. Paul, Minn. A. Healthy Metropolitan bent. B. Healthy Woodhill bent.

"Escape-grass" plants were compared with those of healthy Metropolitan, and it was found that they were of the same type as those in the recovered turf, with the longer root system and bunchy growth form.

Some experiments were made on the effect of fertilizers on the diseased plants. Applications of $(NH_4)_2SO_4$, KH_2PO_4, KNO_3, and H_3BO_3 were made and the plants watched over a period of 6 weeks. In no case was there any sign of recovery, and the death of these experimental plants, together with heavy falls of snow, vitiated the chance of obtaining new material.

DISCUSSION

No causal organism could be isolated from diseased plants. This would indicate that the disease is non-parasitic in origin, a conclusion supported by a great deal of circumstantial evidence. In the first place, neither mercurials nor copper sulphate had any effect in checking the incidence of the disease, and as these chemicals, especially the mercury chlorides, are extremely active fungicides, some weight must be given to this finding. A second factor was the fact that the disease could appear over a very wide range of climatic, topographic, and edaphic conditions. In the usual fungal diseases there are definite factors which influence the attack, *e.g.*, Dahl demonstrated that low temperatures were necessary for the snow mould (*Fusarium nivale*) (3), while Erwin (4) and Coons (2) showed that the presence of epidemics of late blight of potato was dependent on the interaction of temperature and moisture. The degeneration, however, appears over so wide a range of conditions that it seems unlikely that it is caused by a parasite.

There are many factors that cause non-parasitic diseases, but it has been shown above that moisture and soil aeration do not appreciably affect the disease under consideration, so it is concluded that these factors can be eliminated.

In a recent paper, O'Brien and Dennis (8) showed that the difference between susceptible and resistant varieties of swedes to boron deficiency was related to root length. They state: "It appears that the resistant varieties possess a much greater root system than do non-resistant varieties." It is evident, therefore, that there are very good reasons for arguing that the degeneration is due to a deficiency of some minor element or elements in the soil, as the analogy between the swedes and the Metropolitan and Woodhill bents is very obvious.

The extreme density of the turf of Metropolitan bent, coupled with the very short root system, would tend to make it more prone to deficiency diseases than a plant of the Woodhill type. The roots are so small and short that they derive their mineral nutriment from a comparatively small area of soil, and the point is further emphasized by the thickness and density of the turf by reason of which only a small part of the root system will penetrate to the soil. This being the case, the major portion of the root will be growing in the débris of grass clippings and roots, which will not be subject to the normal mineralizing action of the bacteria, as this action is an oxidation process (10) and the circulation of oxygen in such a dense turf is very poor.

This poor circulation of oxygen can be seen in another way. The root of Metropolitan bent if examined *in situ,* can be seen often to turn up at the tip. It is thought that this apparent negatively geotropic response is in reality a positively aerotropic one and is due to the lack of oxygen mentioned above. The benefit of the "spiking" in loosening the surface of the green would then be explained on this basis.

Applications of artificial fertilizers might replace a little of the deficient elements; but Hurst (5) has shown the extreme purity of the modern artificial products in which there are barely traces of the minor elements. It is concluded, therefore, that this degeneration of Metropolitan is probably a deficiency disease, the exact nature of which is not yet fully understood.

SUMMARY

The symptoms of a new disease of Metropolitan bent are: Death of the plants over large areas of the green, with the death of the leaf beginning at the tip. Typical of the disease is the escape of a few plants of a lighter green in the diseased areas.

The disease cannot be controlled by mercurials, aeration, or alteration of the water schedule.

The susceptible variety is characterized by very dense turf formation and extremely short roots, while the escaping plants and resistant varieties have much longer roots.

From an analysis of the evidence the disease is thought to be caused by the deficiency of one of the minor elements necessary to the growth of the plant.

FORMERLY, UNIVERSITY FARM, ST. PAUL, MINNESOTA;
NOW, WEST OF SCOTLAND AGRICULTURAL COLLEGE,
AUCHINCRUIVE, BY AYR., SCOTLAND.

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FIELD STUDIES ON CONCENTRATION OF BENZOL VAPORS AS USED TO CONTROL DOWNY MILDEW OF TOBACCO[1, 2]

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RUTH McLEAN AND P. M. GROSS

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INTRODUCTION

A previous report[3] shows that benzol, C_6H_6, can be effectively employed in the southeastern United States for the control of tobacco downy mildew. It seems desirable to gain a better understanding of the basic principles and factors involved in the use of benzol, since empirical methods were used in the experiments upon which this report was based and since the control of this disease is an important matter in tobacco culture. Preliminary experiments were, therefore, first conducted in the laboratory under controlled conditions to determine the minimal concentrations of benzol vapor that are toxic to tobacco seedlings and to the downy-mildew fungus. On the basis of these findings[4] further experiments were instituted involving varying conditions in seed beds, as a means of studying the factors that modify toxic concentrations of benzol vapors therein. These field studies necessitated trying to establish, first of all, whether benzol can completely prevent infection by the downy-mildew pathogen, or check the disease, once it has been initiated, under environmental conditions different from those that obtained during previous studies. It seems reasonable to believe, if this could be accomplished, that one should be able to determine (a) the volume-percentage concentration of benzol vapors in seed beds that prevent and control downy mildew; (b) the proper ratio between area of evaporators and area of seed bed; (c) the relative efficiency of various types or kinds of evaporators; (d) the modificatory influence exerted by perviousness of seed bed covers to benzol vapor and to meteorological water; (e) the effect of moisture on the covers and on the foliage on retention of effective vapor concentrations within seed beds; (f) the modifications in rate of volatilization induced by mixing benzol with lubricating oils; and (g) the effect of temperature on vaporization of benzol. Rather voluminous data bearing on these matters have been secured. From among these data representative

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[1] Cooperative investigations conducted by the Virginia Agricultural Experiment Station and Duke University.

[2] Special thanks are due Mr. E. G. Moss, Tobacco Experiment Station, Oxford, N. C., for his whole-hearted cooperation.

[3] McLean, Ruth, F. A. Wolf, F. R. Darkis and P. M. Gross. Control of downy mildew of tobacco by vapors of benzol and of other organic substances. Phytopath. **27**: 982–991. 1937.

[4] Pinckard, J. A., F. A. Wolf, Ruth McLean, F. R. Darkis and P. M. Gross. Laboratory studies on the toxicity of benzol vapors to tobacco seedlings and to *Peronospora tabacina*. Phytopath. **29**: 177–187. 1939.

[VOLUME 29, NUMBER 2, FEBRUARY, 1939]

portions have been chosen for presentation as a further contribution to our knowledge of the use of benzol in the control of the tobacco downy mildew.

METHODS AND MATERIALS

Three different locations were chosen for the field work: One in southeastern North Carolina in the vicinity of Lumberton, one in the north central region of the State near Oxford, and the other in southern Virginia, in the vicinity of Chatham. By so doing, varying climatic conditions were encountered and the duration of the investigation was extended over a period of about 2 months.

Seed beds tightly framed with boards were available in each location. Some of the beds made by cooperating growers were framed with poles. The areas of these experimental beds varied in size from 4 to 100 square yards. All beds were prepared in the customary manner and were kept covered with ordinary tobacco-seed-bed cloth until medication with benzol commenced.

About 25 of the more common flue-cured varieties of tobacco were used, as were also varieties of Turkish, Burley, and several hybrids. Details regarding these varieties are omitted for the reason that none exhibited appreciable differences either in susceptibility to the downy-mildew fungus or to injury by benzol vapors.

Commercial 90 per cent benzol[5] was employed throughout these studies. It was vaporized by means of several kinds of metal evaporators, Fig. 1, placed at predetermined distances throughout the seed beds. They included pans, troughs, tubes and devices containing wicks.[6] The pans were supported on racks and were protected with suitable canopies to deflect the rain. The troughs, also elevated on supports, were covered with galvanized iron roofs. In one kind they fitted closely; in another, a substantial space was left between the edge of the troughs and the overhanging roofs. The tubes were water pipes with a series of closely spaced quarter-inch perforations along the upper side. Other evaporators consisted of metal containers fitted with cotton wicks that could be adjusted to expose the desired areas for evaporating. These containers were connected in series by metal tubes and were filled by gravity from a common reservoir.

The procedure used in vaporizing benzol consisted in placing measured quantities of commercial benzol in evaporators usually at about 7:00 p.m. In some of the evaporators the benzol was mixed with lubricating oil in order to retard the rate of evaporation. Preliminary experiments had shown that oils, regardless of their viscosity and specific gravity, are not significantly different in their ability to retard the volatilization of benzol.

Special seed-bed covers[7] were used during fumigation and were removed

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[5] Benzol was supplied through the courtesy of the American Oil and Supply Co., Newark, N. J. The specifications are contained in another report (4).

[6] Supplied through the kindness of Mr. E. C. Beinhart.

[7] The Agricultural Adjustment Administration, Washington, D. C., courteously supplied the covers.

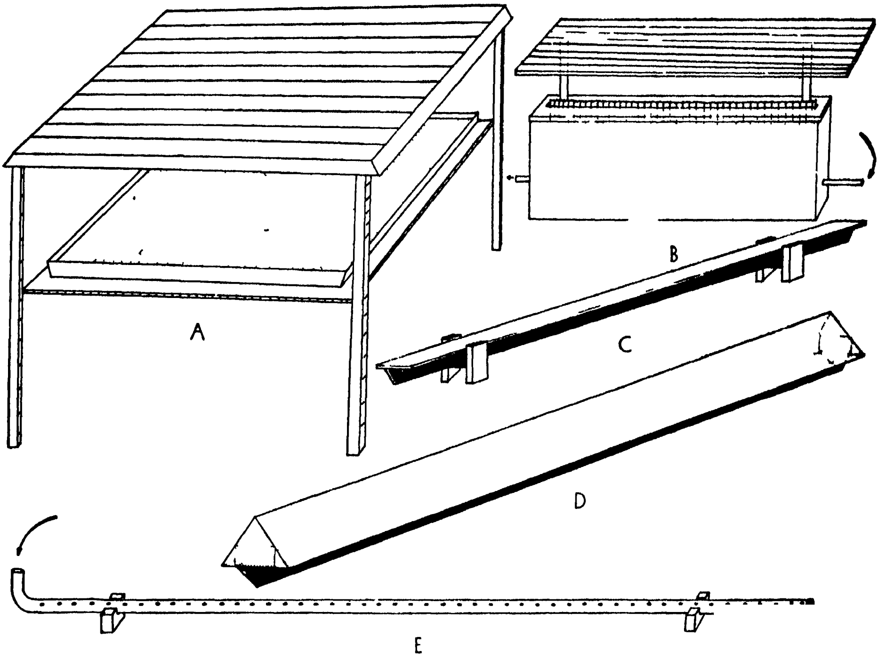

FIG. 1. Sketches of different kinds of appliances used to vaporize benzol. A. Pan type in which pan is placed on shelf about 8 in. from the ground. A roof of wood or galvanized iron shields the pan against rain. B. Wick evaporator, covered by a roof. These metal containers may be connected in series and filled from a common reservoir placed outside the bed. Rate of evaporation regulated by varying the area of wick exposed. C. Angle-iron trough, covered with a strip of galvanized iron and supported by stakes. Angle iron of type used in structural work. Cover may be lifted and benzol poured into trough. D. Galvanized-iron trough, cover of similar material elevated above trough by metal ends. Such troughs may be placed on wooden supports. Cover detachable at one end to facilitate filling. E. Iron piping with $\frac{1}{4}$ in. holes drilled along upper side. Open end extends outside seed bed to facilitate filling. Pipe seated either on seed-bed frame or on special bases.

during the daytime. These covers, except in one series of experiments, consisted of unbleached sheeting having 64 warp and 64 woof threads per inch and a weight of 2.68 yards per pound. In the exceptional series, covers of varying specifications were used to determine their perviousness to benzol vapors and to water.

In order to facilitate sampling the atmosphere within the beds to determine its benzol vapor content, copper tubes were installed. The inlet of each tube was placed at a uniform distance from an evaporator, the other end being outside the bed. Each tube was equipped with a brass coupling to facilitate attachment to the instrument (Fig. 2) employed to withdraw samples of benzol-air mixtures from the beds when measuring their benzol content. This instrument was a Mine Safety Appliance Combustible Gas Indicator.[8] Benzol vapors, aspirated into the instrument, passed over an electrically heated catalytically activated platinum filament, thus burning the benzol. The filament comprised one arm of a Wheatstone bridge circuit.

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[8] Manufactured by the Mine Safety Appliance Co., Pittsburgh, Pa.

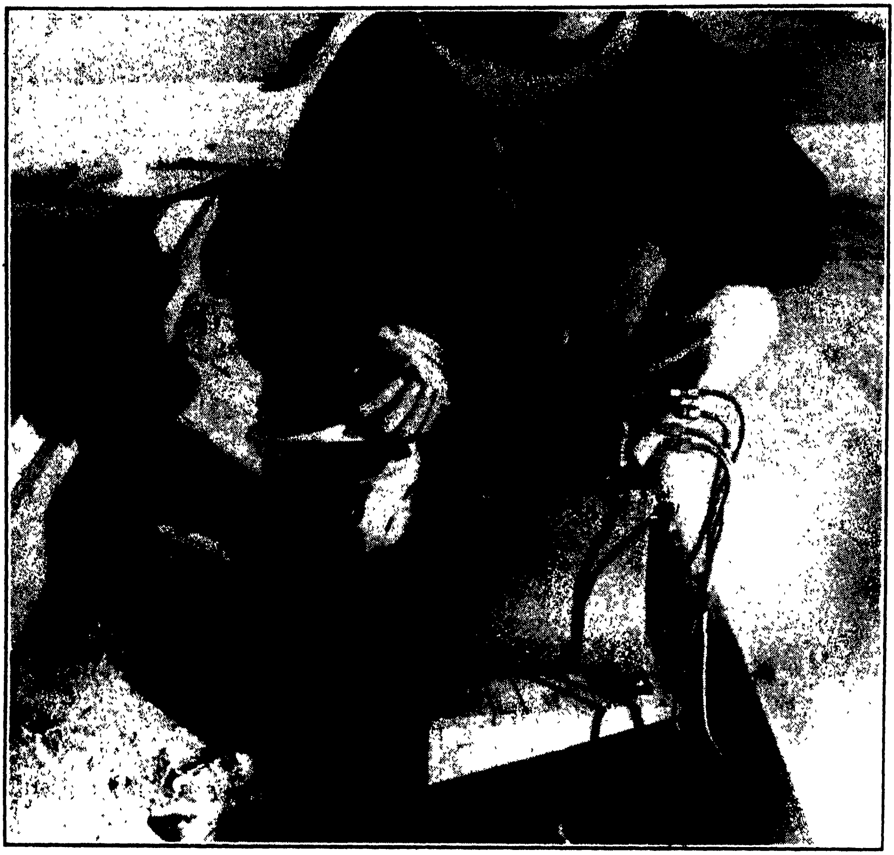

FIG. 2. Mine Safety Appliance Combustible Gas Indicator, used for analysis of benzol vapors in tobacco seed beds. Benzol vapor was aspirated from the bed through copper tubes extending into seed bed.

The change of resistance of the filament caused by the combustion of the benzol on its surface produced electrical unbalance of the bridge circuit. The extent of this unbalance was indicated by a milliammeter calibrated to read directly in volume percentage of benzol in the sample. To insure reliability, the M. S. A. Indicator was calibrated at intervals. This was accomplished by introducing measured quantities of benzol into a carboy of known capacity. After thorough agitation, samples of the benzol-air mixture from the carboy were analyzed by the M. S. A. Indicator and were checked by the combustion method of analysis, previously described.[9] From the data obtained by a series of analyses made in this manner a curve was plotted of the meter readings against calculated benzol concentrations in the carboy.

As a further check on the accuracy of the M. S. A. Indicator, samples of benzol-air mixtures from the seed beds were collected in evacuated containers for analysis by the combustion method. These samples were withdrawn immediately after analysis had been made by the M. S. A. Indicator. Typical comparative analyses are shown in table 1.

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[9] See footnote 4.

TABLE 1.—*Comparative analyses of benzol vapors in seed beds made by means of the chemical method and of the M. S. A. Indicator*

Volume of gas collected	Benzol found by chemical analysis	Benzol found by chemical method	Benzol found by M. S. A. Indicator
Ml.	*G.*	*Vol. percentage*	*Vol. percentage*
2280	0.0032	0.044	0.030
3910	0.0104	0.082	0.095
3915	0.0468	0.367	0.380
3903	0.0416	0.326	0.320
3900	0.0416	0.326	0.330
3900	0.0390	0.306	0.420
1940	0.0312	0.492	0.490

EXPERIMENTAL RESULTS

Prevention and Control of Downy Mildew in Seed Beds

Complete prevention of tobacco downy mildew was accomplished during the past season in each of 3 selected localities. The portion of the seed bed near Lumberton in which this was done comprised 64 sq. yd. with a control area of 20 sq. yd. Complete control was secured on 61 sq. yd. near Oxford with 20 sq. yd. serving as a control. The total area of successfully treated beds near Chatham was 752 sq. yd. with 1514 sq. yd. of control beds.

On the basis of previous studies on the epidemiology of downy mildew,[10] the conditions at Lumberton throughout these experiments were continuously favorable for a serious epidemic. Treatment was begun when the outbreak of downy mildew occurred in the immediate vicinity of the experimental beds, at a time more than a week in advance, however, of the occurrence of the disease in the control beds. The disease also appeared on the same date in those portions exposed to amounts of benzol equivalent to 25 ml. per sq. yd. per application, employing evaporators with areas 1/100 and 1/144, respectively, as large as those of the beds. The disease never attained epidemic proportions in areas treated with these rates of application, although it continued to develop on seedlings most distant from the evaporators. It became epidemic, however, in the control portions and remained entirely absent in the area in which applications of 25 ml. per sq. yd. were made with evaporators 1/72 of the area of the seed bed.

Downy mildew appeared a day or two prior to initiation of treatments at Oxford. During the period of these experiments the precipitation was insufficient to favor satisfactory growth of the seedlings, necessitating watering the beds. Even though prevailing maximum temperatures were rather unfavorable, minimum temperatures favored sporulation every night throughout the duration of the treatments. In order to bring downy mildew under control in certain of the beds during such weather conditions, it was found necessary to use 4 times the usual amounts of benzol for a single application. Sporulation did not occur subsequently in portions to which this treatment was given if, thereafter, they received the usual treatment of 25 ml. of benzol per sq. yd. per application, employing evaporators with areas 1/72 the size of the seed bed.

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[10] Dixon, L. F., Ruth A. McLean, and F. A. Wolf. Relationship of climatological conditions to the tobacco downy mildew. Phytopath. 26: 735–759. 1936.

Near Chatham several growers supplied the major proportion of the successfully treated beds. Although the weather was dry for most of the period during which treatments were made, sporulation occurred every morning in varying amounts. Complete prevention of the disease was obtained in 560 sq. yd. of seed beds, operated by growers, using 25 to 50 ml. of benzol per. sq. yd. per application in pan evaporators 1/72 to 1/89 of the area of the seed bed. For the most part, treatment of growers' beds was begun soon after the disease appeared, and was continued until the seedlings were ready to transplant. Figure 3 shows the appearance of a typical treated and nontreated area of the grower's seed bed several days after the disease had been checked in the fumigated portion. In several instances unsuccessful attempts were made to control the disease by using 12.5 ml. of benzol per sq. yd., with an evaporating area 1/134 that of the seed bed. Similar results also were obtained at the Experimental Station, both in seed beds in which this rate of application per unit was used and in those in which twice the amount of benzol was applied. Irrigation of these beds

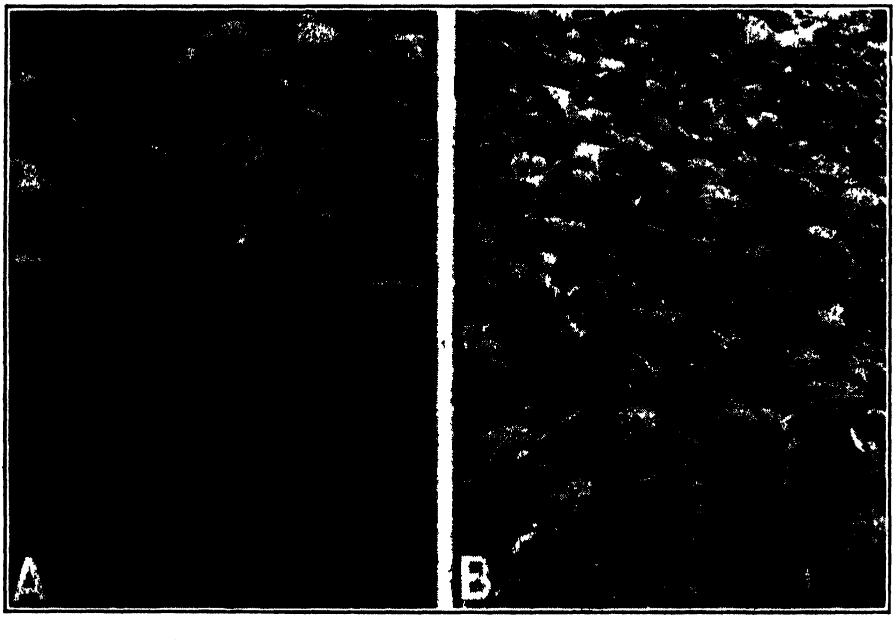

FIG. 3. A. Appearance of seed bed of grower several days after downy mildew was checked by use of benzol vapor. B. Nontreated portion of same bed, separated from treated area by a ¾ in. board, April 30, 1938.

favored development of the disease, whereupon 100 ml. of benzol per sq. yd. was applied on each of 2 successive nights, in pans having an evaporating surface 1/72 of that of the bed. As a consequence, sporulation was checked and treatments were discontinued. Sporulation was resumed in these beds however, 6 days later, when fungicidal applications were repeated with the result that the disease was again checked.

In 2 experiments involving tolerance of diseased tobacco seedlings to benzol vapors, a single application of 250 ml. per sq. yd. was made in pan evaporators with an area 1/10 that of the bed. Temperatures ranged from 11.7° to 28.3° C. The covers were kept moistened and the volume percentage concentration of benzol vapor was maintained between 0.63 and 1.6 for 12 hours. In neither experiment were the seedlings injured by this treatment, but the pathogen was killed. The disease reappeared in these beds a week later, however. In another experiment, both the amount of benzol and the area of evaporating surface were doubled. The cover in this case was water-proof, and the temperatures ranged from 13.9° to 25.5° C. The bed was watered lightly immediately prior to applying the benzol. Treatment was begun in mid-afternoon and continued for 14 hours. The benzol vapor concentration reached 2.0 volume per cent within an hour. As a result the foliage was severely injured, but eventually the seedlings recovered.

CONCENTRATIONS OF BENZOL VAPORS WITHIN SEED BEDS

While valuable data concerning the concentration of benzol vapors were obtained from those beds in which complete prevention was accomplished, equally valuable data were procured in each locality by use of seed beds in which only a measure of control was secured. The M. S. A. Indicator was employed under a variety of conditions in attempts to correlate the benzol vapor concentrations found in seed beds with treatment practices. Observations on the progress of downy mildew were used to interpret these data. At Lumberton, during 9 nights, 289 measurements of benzol vapor concentration were made with the M. S. A. Indicator, 569 at Oxford during 9 nights, and 385 at Chatham during 4 nights. Manifestly, it is beyond the compass of this report to present all of these data; consequently, representative portions only are presented.

In a general way these measurements show that fungistatic action is secured when benzol-vapor concentrations in the atmosphere range from 0.025 to 0.10 per cent by volume during the nightly periods of treatment. Minimal volume percentage concentrations of benzol vapor for fungicidal action within seed beds are approximated within the range 0.40 to 0.50. When a volume per cent benzol vapor concentration of the atmosphere in seed beds within the range of 2.0 to 2.5 is reached injury results, varying in amount from slight necrosis to serious damage.

In the studies that follow it should be kept in mind that the treated seedlings were later used in the field or for agronomic investigations, so that experimentation on mechanical and environmental factors influencing the concentration of the vapor phase of benzol interfered in no appreciable way with the health of the seedlings. Manifestly, investigation of these factors should show how each one modifies the rate at which benzol is evaporated and the degree to which the vapors are retained inside the beds.

Influence of Types of Evaporators

Numerous measurements were made of benzol vapor concentrations at varying distances from the evaporators of the kinds shown in figure 1. Except for the wick-type and the perforated tubes, they were all constructed of a size to provide a ratio of 1 : 72 between area of evaporating surface and that of bed. In the case of the wick-type and of the perforated-tube evaporators, the design of construction permitted the evaporation of a minimum of 2.5 ml. of benzol per hour. Choice of kind of evaporators would seem to be governed by cost and convenience, since equally effective control of downy mildew was accomplished with each.

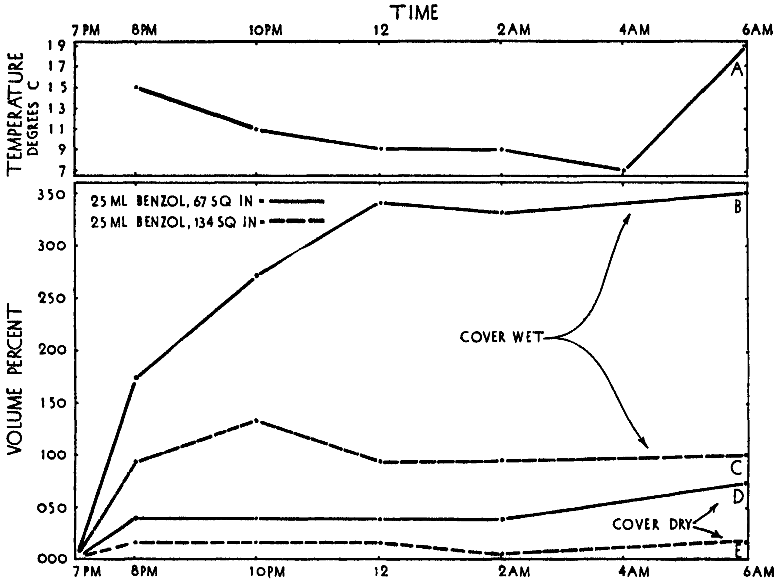

FIG. 4. Benzol vapor concentrations in seed beds. Pan evaporators with area equivalent to 1 sq. in. per 67 sq. in. of bed area, and 1 to 134, were used. Application, 25 ml. of benzol per sq. yd. A. Temperature during treatment. B–E. Volume-percentages of benzol at times and under conditions indicated.

Influence of Variation in Ratio of Evaporator Area to Seed Bed Area

As previously indicated in this report, if the ratio between area of evaporating surface to seed bed is less than a certain amount, the amount of benzol applied being kept constant, control of downy mildew was not accomplished. To find a reason for successful control with one ratio and failure with another, measurements of benzol-vapor concentrations in otherwise comparable beds were made. In one the evaporating area was 1/67 that of the bed and in the other 1/134. In each an amount of benzol equivalent to 25 ml. per sq. yd. was applied. Typical results are shown graphically in figure 4. When figure 4, D and figure 4, E are compared it is apparent, as could have been anticipated, that the higher vapor concentrations are found in beds with the greater ratio of evaporator area to seed-bed area. When figures 4, D and E are compared with figures 4. B and C, a measure may be had of the extent to which moisture on the covers modifies the escape of vapors from the beds. The concentrations shown in figure 4, C are within the fungistatic range, indicating that control may be accomplished in seed beds with evaporators having an area 1/134 of that of the seed bed, provided the covers are kept moist. The concentrations of vapors represented in figures 4, D and E are, however, too low to be of value in control.

Influence of Type of Seed-bed Cover

The openness of texture of cloth may be stated in terms of the number of threads in the warp and woof and the area of the cloth per pound.[11] Their effectiveness as seed-bed covers is, however, more directly related to their ability to modify the passage of water or of fungicidal vapors. Determinations of this ability were made by stretching samples of several cloths tightly over the sharp edge of containers having a surface area of 30.68 sq. in. An uncovered container served as a check. All were set out of doors under similar conditions. Measurements for 24-hour periods of exposure were made at 6 p.m. on 9 days during which there was rainfall of 0.04 to 0.9 inch. The comparative penetrabilities of the covers are shown (Table 2) by comparing the average total amount of water collected in each case minus that evaporated with the average total collected from the uncovered control. The uncovered control is regarded as standard, or 100 per cent.

TABLE 2.—*The relative efficiency of various grades of seed-bed covers in modifying the passage of meteorological water*

Kinds of covers	Percentage water remaining
Uncovered control	100
Warp 24, woof 20, wt. 17.0	96
Warp 32, woof 28, wt. 13.0	93
Warp 44, woof 40, wt. 8.2	83
Warp 48, woof 48, wt. 7.15	67
Warp 56, woof 60, wt. 4.0	40
Warp 48, woof 48, wt. 4.0	17
Warp 64, woof 64, wt. 2.75	15

From the above table it is apparent that the cloth possessing the greatest number of warp and woof threads per inch is least pervious. While these values do not show separately the amount of rain that entered through each cloth and the amount of water that evaporated through each, they do indicate their comparative abilities to govern the passage of water.

The ability to retain benzol vapors by cloths of these same kinds was tested by analyses of the benzol content of seed-bed atmospheres. Fifty ml. of benzol per sq. yd., mixed with 500 ml. of oil, were placed in pan evaporators of 1/64 the area of the bed. The foliage of the seedlings was sprinkled with water and the covers were moistened after being fastened in position. Results of samplings, during one of the nights, together with temperature readings, are shown in figure 5. It is readily apparent from these data that

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[11] Weight taken as square yards per pound.

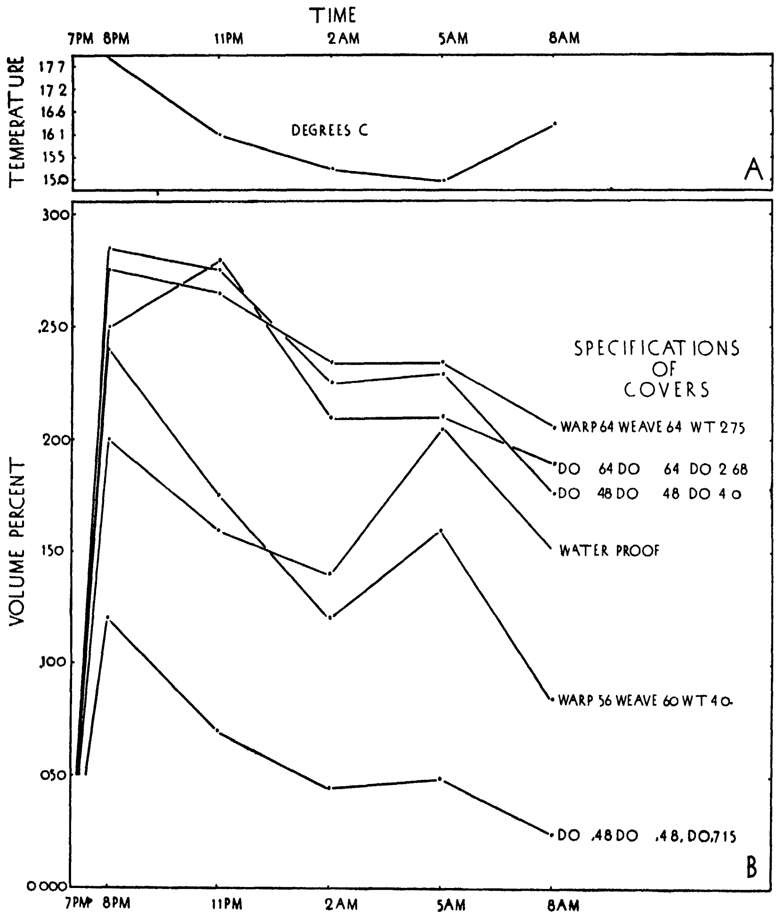

FIG. 5. Benzol-vapor concentrations in seed beds with wet covers of varying warp, woof, and weight. Benzol, 50 ml. mixed with 500 ml. of oil in pan evaporators. Area of evaporators equivalent to 1 sq. in. per 64 sq. in. of bed area. A. Temperature at times of analysis. B. Volume-percentage concentrations found.

the cloths differed in ability to impede the escape of benzol vapors much as they did the passage of rain. The densely woven cloths were the most retentive of benzol vapor, while the loosely woven ones were highly inefficient. It is thus apparent that the area of the cloth per pound (usually spoken of as its weight) bears an inverse relation to its vapor retaining efficiency. It may be pointed out that the warp and woof of a cloth together with its weight should be specified if significant comparisons are to be made in trials of this kind.

The water-proof cover used was reputed to be gas-proof. Numerous measurements of benzol vapor concentrations made under such cover showed it to be consistently less efficient than ordinary densely woven, moistened covers.

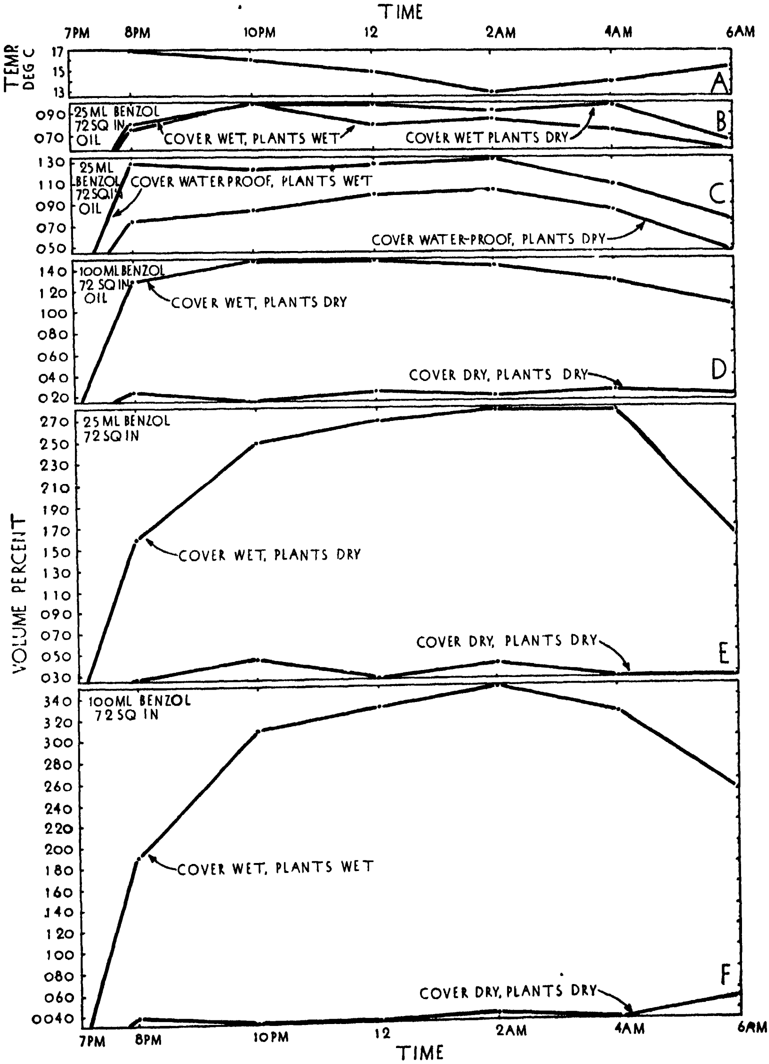

FIG. 6. Benzol-vapor concentrations in seed-bed atmospheres graphically presented. Moisture conditions indicated. Covers either 64 warp, 64 woof, 2.68 yd. per lb., or water-proofed. Pan evaporators with area equivalent to 1 sq. in. per 72 sq. in. of bed area. A. Temperature at times of analysis. B to F. Volume-percentage concentrations: B, using 25 ml. of benzol in 500 ml. of oil; C, using 25 ml. of benzol in 500 ml. of oil, under water-proof cover; D. using 100 ml. of benzol in 500 ml. of oil; E. using 25 ml. of benzol; F, using 100 ml. of benzol.

Influence of Oil

As mentioned earlier in this report the viscosity and specific gravity of lubricating oil does not appear to bear any significant relation to the rate of evaporation of benzol when benzol and oil are mixed. It seemed pertinent, however, to find to what degree oil modified the benzol vapor concentration when evaporation from benzol alone was compared with evaporation from benzol-oil mixtures. Measurements were made of such concentrations in numerous cases, by means of the M. S. A. Indicator. Typical results are graphically recorded in figure 6.

Under comparable seed-bed conditions, substantially higher benzol vapor concentrations were found (Fig. 6) in beds in which the benzol was used alone than in those in which benzol-oil mixtures were employed. The use of oil, therefore, appears to retard the rate of evaporation, and, in so doing, tends to maintain the benzol-vapor concentration at a more uniform level. Under certain conditions this may be a very distinct advantage. Until more is known on the duration of the period for maintaining the toxic concentrations, the use of oil cannot be said to be necessary.

Influence of Temperature

Chemically pure benzol has a melting point of 5.4° C. and a boiling point of 80.4° C. Vapor pressures between these extremes show a curvilinear relationship, the greatest change in pressure per unit change in temperature occurring in the higher temperature ranges. Within the range of temperatures that obtain while tobacco seedlings are being grown, one would anticipate finding that differences in the volume-percentage concentration of benzol vapor in the air of beds should be correlated with differences in temperatures. Accordingly, tests were made of benzol-vapor content of the atmosphere in beds on a cold night and on a warm one, under conditions otherwise entirely comparable (Fig. 7).

The temperatures during the early period of treatment on these 2 nights differed by approximately 10°. Since the vapor pressure of benzol is ap-

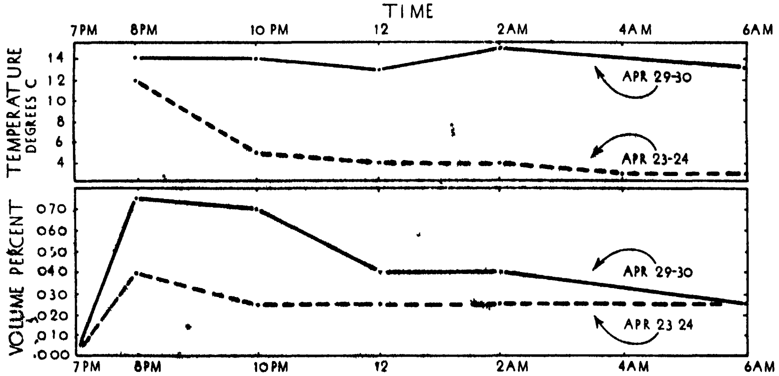

FIG. 7. Benzol-vapor concentration in the same seed bed, other conditions being identical, under 2 temperature conditions, a warm night and a cool night.

proximately twice as high at the higher as at the lower temperature, the comparative effects of temperature on evaporation rate and on concentrations (Fig. 7), are of the order of magnitude anticipated. These relationships were maintained during most of the 2 nights compared. While these data appear to indicate that temperature is a factor in modifying the efficiency of treatment with benzol, temperature is really of minor importance in field practice in comparison with the large effects produced by the presence of moisture on the covers and on the seedlings (*cf.* Fig. 6).

Influence of Moisture

The studies involving the influence of moisture on the concentration of vapor of benzol within beds aimed to contrast the measurements of the concentrations found when the covers were wet with those obtained when the covers were dry. Contrasting measurements also were made of concentrations occurring when the foliage of the seedlings was wet with those occurring when there was no visible moisture on the leaf surfaces. By using wet or dry covers and keeping the foliage wet or dry, other combinations were effected. Representative comparative measurements of volume-percentage vapor concentrations under varying moisture conditions at stated temperatures are shown in figure 6, A–F.

The outstanding feature presented by figure 6 is that markedly higher concentrations of benzol vapor always occur within beds whose covers are wet. If the covers are dry they exert comparatively little influence in helping to confine the vapors. Covers are essentially benzol-vapor-tight so long as they remain wet. It is also apparent that the cover processed with a collodion-like material to make it water-proof, is less efficient in confining benzol, as shown in figure 6, C, than the non-water-proofed covers, provided the latter are wet with water. In all cases the concentration of benzol vapor is greater if the foliage of the seedlings is wet than if it is dry (Fig. 6, B, C, and F). The measurements also are consistently higher, as could be expected, in beds in which the larger amounts (Fig. 6, F compared with Fig. 6, E) of benzol per unit area of seed bed are permitted to be volatilized.

The data graphically shown in figure 6 might have given a more complete picture of the water relationships in modifying concentrations of benzol vapor within the seed beds if they had been supplemented by a series of analyses under identical conditions showing the benzol concentrations in the water on the foliage. In lieu of this, certain analyses were made of vapor in water on the foliage and on the covers. The results of these analyses are shown in table 3.

The important feature established by the data in table 3 is that benzol, if vaporized in tobacco seed beds, can be demonstrated as present in the water, both on the foliage and on the covers. To facilitate the comparison of the relative concentrations in the atmosphere and in the water layers, the concentrations in the latter have been expressed in terms of the volume percentages of benzol vapor that would be in equilibrium with the concen-

TABLE 3.—*Benzol vapor content of water in seed beds on seedlings and on seed-bed covers*

Sample No.	Place	Date	Hour	Temperature ° C.	Source of sample from:	Weight of sample	Benzol in liquid found by combustion	Benzol in atmosphere M. S. A. Indicator reading	Vapor concentration C_6H_6 in equilibrium with concentration in water[a]	Remarks
						G.	*G.*	*Vol. percentage*		
1	Lumberton, N. C.	4–1–38	10: 20 p. m.	18.9	Leaves	8.9765	.0130	0.49	7.8	Pan evaporators, 1/72 ratio, benzol 25 ml. per square yard, rain falling.
2	Lumberton, N. C.	4–1–38	10: 25 p. m	18.9	Leaves	7.8049	.0104	0.49	7.1	Do
3	Lumberton, N. C.	4–1–38	10: 30 p. m.	18.9	Underside of cover	6.7912	.0130	0.49	10.2	Do
4	Oxford, N. C.	5–1–38	2: 15 a. m.	9.5	Leaves	8.5833	.0000	0.00	0.00	Sample from bed used as control.
5	Oxford, N. C.	5–1–38	2: 20 a. m.	9.6	Outside of cover	7.6797	.0000	0.03–0.08	0.00	From bed with wick evaporators
6	Oxford, N. C.	5–1–38	3: 00 a. m.	9.5	Leaves	4.0892	.0052	0.03–0.08	3.4	From bed with wick evaporators.
7	Chatham, Va.	5–9–38	11: 15 p. m.	9.0	Leaves	11.5464	.0052	0.13–0.17	1.4	Cover wet, foliage wet, benzol 100 ml. plus 500 ml. oil, evaporators 1/67 ratio.
8	Chatham, Va.	5–9–38	11: 20 p. m.	9.0	Leaves	12.1393	.0026	0.05–0.09	0.6	Water-proof cover, foliage wet, benzol 25 ml. plus 500 ml. oil, evaporators 1/67 ratio.
9	Chatham, Va.	5–9–38	11: 30 p. m.	9.0	Underside of cover	11.3706	.0156	0.05–0.09	4.2	Do
10	Chatham, Va.	5–9–38	11: 35 p. m.	9.0	Leaves	9.1188	.0000	0.00	0.00	Do

TABLE [illegible].—(*Continued*)

Sample No.	Place	Date	Hour	Temperature °C.	Source of sample from:	Weight of sample	Benzol in liquid found by combustion	Benzol in atmosphere M. S. A. Indicator reading	Vapor concentration C_6H_6 in equilibrium with concentration in water[a]	Remarks
11	Chatham, Va.	5–9–38	3: 05 a. m.	7.0	Leaves	13.7224	.0078	0.08–0.10	1.5	Cover wet, foliage wet, evaporators 1/67 ratio, benzol 25 ml. plus 500 ml. oil.
12	Chatham, Va.	5–9–38	3: 00 a. m	7.0	Outside of cover	8.5327	.0000	0.08–0.10	0.00	Do
13	Chatham, Va.	5–9–38	3: 10 a. m.	7.0	Underside of cover	8.7886	.0052	0.08–0.10	1.1	Do
14	Chatham, Va.	5–9–38	3: 15 a. m.	7.0	Leaves	11.8692	.0078	0.38	1.8	Cover wet, foliage wet, evaporators 1/10.8 ratio, benzol 250 ml.
15	Chatham, Va.	5–9–38	4: 00 a. m.	7.0	Leaves	9.0354	.0065	0.13–0.17	2.0	Cover wet, foliage wet, evaporators 1/64 ratio, benzol 100 ml. plus 500 ml. oil.
16	Chatham, Va.	5–9–38	4: 10 a. m	7.0	Leaves	10.6081	.0052	0.06–0.10	1.3	Plants wet, water-proof covers, benzol 25 ml. plus oil, evaporators 1/67 ratio.

[a] Volume per cent of C_6H_6 vapor, which would be in equilibrium with concentration bound in the H_2O (liquid phase), was calculated as follows:

$$\frac{\dfrac{\text{Wt. of } C_6H_6 \text{ in gms. per liter}}{\text{Sol. of } C_6H_6 \text{ in } H_2O \text{ in gms. per liter}} \times \text{Vapor pressure of } C_6H_6}{\text{Atmospheric pressure}} \times 100 = \text{Volume per cent.}$$

trations found in the aqueous layers analyzed. It will be seen that the benzol concentration in the water samples taken from within the beds, was in all cases and at all times greater than that in the atmosphere of the bed. Samples 5 and 12, taken from the upperside of the covers, showed no benzol in the water film, although the Indicator showed it to be present within the atmosphere inside the bed. Conditions were ideal during the night in which samples 1, 2, and 3 were taken, for retaining the vapors within the beds.

The times of collection were sufficiently varied in these experiments to indicate that effective fungicidal concentrations of benzol vapor in water could be expected to exist throughout the entire night in treated beds. If a correlation exists between vapor concentration in the atmosphere and in the water, these data are too meagre to demonstrate it. All data, however, not only in these but in all other experiments incline us to the opinion that moisture relations constitute the most important factor in fumigation with benzol.

DISCUSSION

Experimentation performed previous to the present studies to prevent or to control tobacco downy mildew in seed beds has not yielded data on the definite concentrations of benzol vapor necessary for this purpose. The concentrations of benzol vapor in the atmosphere of seed beds that have been found to be toxic to tobacco seedlings or to the tobacco-downy-mildew pathogen are of the same order of magnitude as those found to be toxic under laboratory conditions.[12] Since such concentrations are difficult to maintain in seed beds for periods comparable with those maintained in the laboratory, data are still lacking on the duration of the period of treatment as an interdependent factor in fumigation with benzol.

The range in limits of toxicity established by these measurements of vapor concentrations in beds shows that the downy-mildew fungus is killed at concentrations that are approximately one-sixth of those causing injury to the seedlings, and that control of the disease can be maintained by use of concentrations one-thirtieth of those causing injury to the seedlings. These differences augur well for the safe use of benzol as a fumigant with tobacco plants

Data have not been available previously to aid in interpreting the interrelationship of the several mechanical and environmental factors involved in the successful use of benzol. In interpreting data obtained by measuring the benzol-vapor concentrations in seed beds, moisture conditions appear to constitute the most important factor involved in developing and in maintaining concentrations that are fungistatically and fungicidally effective. This conclusion supports the explanation developed from laboratory studies[12] of the mechanism of the toxic action of benzol, wherein it was pointed out that benzol in its vapor phase enters rather quickly into aqueous solution and that water constitutes the vehicle through which the benzol vapor acts.

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[12] See footnote 4.

Under usual conditions in seed beds, at nightfall water vapor is condensed as dew that accumulates both on the bed covers and on the seedlings. At present too little is known regarding concentrations of benzol vapor in this accumulated dew. It appears to be desirable to know what concentrations occur in the dew within the beds throughout the night and, at the same time, the vapor concentrations in the atmosphere of these beds.

Even though the limits of toxicity of benzol vapor are established from measurements of the content of benzol vapor in the atmosphere of beds, these concentrations do not actually represent the ones effective in producing injury to the seedlings or in killing the pathogen. As indicated by the findings in table 3, the effective concentrations, in all likelihood, in the free water and perhaps in the cell sap, are much in excess of these values. Under practical conditions, measurements of benzol content of the air may serve as a readily determinable index of effective concentrations.

All of the evidence indicates that the vapors escape from the beds essentially as rapidly as the benzol is vaporized, if the covers remain dry at night as may happen in windy weather. If a series of such nights were to prevail during which the temperatures were favorable for sporulation, downy mildew might not be satisfactorily controlled. Under such conditions larger quantities of benzol than normal are required if inhibitory or lethal concentrations of vapor are to be developed. In actual field practice, only an occasional dry, windy night interrupts the sequence in which sufficient moisture is deposited on the covers to be effective in impeding the escape of benzol.

The use of gaseous fungicides that can be disseminated to cover all aboveground parts of tobacco seedlings was indicated as most likely to give control of downy mildew, as previously stated:[13] (a) because complete coverage of the foliage cannot be secured by sprays or dusts; (b) because the infection hyphae may penetrate either leaf surface; and (c) because the leaves rapidly increase in size, making daily application of protectants desirable. For these reasons various volatile substances, among them benzol, were employed before it was appreciated that it is fungicidally effective by virtue of its ability to dissolve in water at the surface of the host or parasite or within the cell constituents.

SUMMARY

These studies with benzol as a fungicidal agent against tobacco downy mildew in seed beds confirm previous experiments in showing that it can be successfully employed for this purpose.

Downy mildew may be completely prevented if benzol fumigation is initiated prior to the outbreak of the disease and is continued throughout the epidemic. If treatment is begun after the outbreak, further progress of infection may be checked.

Even when amounts of benzol lethal to the pathogen are applied it becomes very difficult to injure tobacco seedlings under field conditions, since the limits of toxicity of benzol to the parasite and to the host are widely separated.

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[13] See footnote 3.

In these studies the making of numerous measurements of benzol-vapor concentration was facilitated by use of a Mine Safety Appliance Combustible Gas Indicator.

The volume-percentage concentrations of benzol vapors in the atmosphere of seed beds that are toxic to the pathogen and of those causing injury to tobacco seedlings agree closely with values previously established from laboratory studies.

Among the factors that influence the effectiveness of fumigation with benzol as measured by vapor concentrations are (a) amount of benzol applied per unit area of seed bed, (b) ratio between area of evaporators and area of seed bed, (c) porosity and penetrability of the covers as modified by their texture and by rain or dew on the covers, (d) rate of volatilization, as modified by temperature and as retarded by mixing lubricating oil with benzol, and (e) presence of water on the foliage of the seedlings. Some of these factors are difficultly separable, but attempts were made to evaluate each one.

Moisture on the covers and on the seedlings constitutes the most essential condition in the effective use of benzol in seed beds. This conclusion supports an hypothesis on the mechanism of the mode of action of benzol that had been postulated from the laboratory study of its toxicity.

Although the effective range of benzol-vapor concentrations in the atmosphere of the beds is known, the effective concentrations in the free water on the foliage and in the cell constituents remain unknown. The effective benzol concentrations in water in these situations during fumigation are indicated to be in excess of those in the atmosphere of beds.

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INTERNAL BLACK SPOT OF GARDEN BEET

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INTRODUCTION

During the past several years the crop of garden beet (*Beta vulgaris* L.) grown extensively in Wisconsin for canning has been damaged noticeably by an internal necrosis of the fleshy root. This disease, which is referred to herein as internal black spot, has been noted in other states and its economic importance to the beet canning industry is now generally recognized. The purpose of this paper is to describe the symptoms of the malady as it develops in Wisconsin and to report progress of investigations that have been conducted upon its nature and control.

SYMPTOMS OF INTERNAL BLACK SPOT

Undoubtedly the time of appearance will vary with location and environment, but where the disease has been studied in Wisconsin it usually develops in the latter part of the growing season as the crop is approaching the canning stage. Since the striking and ultimately destructive phases are to be seen in the fleshy root they will be discussed first, and a description of the disease as it affects the foliage will follow.

The internal necrotic areas are irregular in size and shape. They may be confined to the central region, the periphery, or may be scattered promiscuously throughout the fleshy root. The spots are most conspicuous between the prominent rings, which are marked by the heavy-walled vessels formed by the activity of the secondary cambium zones in the pericycle. Undoubtedly their location is influenced by the relative activity of the cambial rings as the fleshy root enlarges, but more study on this point is necessary before any definite statement can be made. The exact point of initiation of the necrosis, whether in the cambium, secondary xylem, or secondary phloem cells has not been ascertained. The necrotic tissue is black, or nearly so, and hard in texture. A thin hand section of an affected root shows that as the necrosis begins to appear it is surrounded by cells of lighter color than the adjacent normal ones, while in older spots a ring of tissue made up of colorless cells surrounds the diseased area. The dead tissue does not desiccate to cause shrinkage and cavity formation, except near the periphery, and the internal spots thus appear as black blotches in the fleshy tissue. In figure 1, A, a cross section of a diseased root is shown from which the red coloring matter was removed

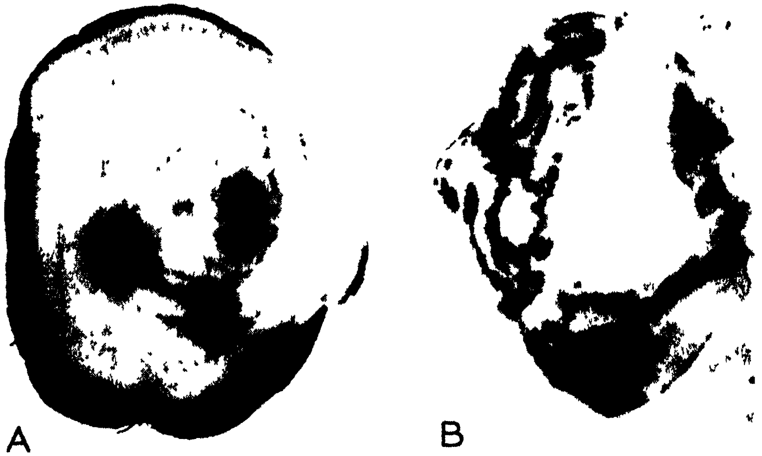

FIG. 1. Internal black spot of garden beet. A. Large, black areas of necrotic tissue between the zones of heavy-walled xylem cells near the center and in one case near the outer surface. B. More or less continuous bands of necrotic tissue located in the region of parenchymatous cells between the zones of heavy-walled xylem.

before photographing. The xylem zones are quite widely separated and the location of the blotches between them is discernible. In this instance three large blotches occur near the center and one near the periphery. In figure 1, B, is another bleached beet from the same lot, in which the necrosis is more widely distributed and less concentrated in separate spots. There is the same tendency, however, to develop most conspicuously between the zones of heavy-walled xylem vessels.

When all of the necrosis occurs well within the periphery there is no evidence of the disease on the surface of the root at harvest. In such cases the detection of internal black spot and the rejection of diseased beets at the cannery can only be accomplished by cutting the roots. When the necrosis forms near the periphery it may commonly be detected at the surface. This is due to the fact that when the breakdown is sufficiently near the outermost cells of the roots a rift at the surface occurs. The entrance of secondary organisms from the soil then adds to the complexity of the picture and surface cankers are formed. In some instances the canker stage may be the most conspicuous feature in a given field, while in other cases the internal unbroken lesions may predominate. In view of the fact that the peripheral lesions are in the region of the most active cells of the pericycle, it is not

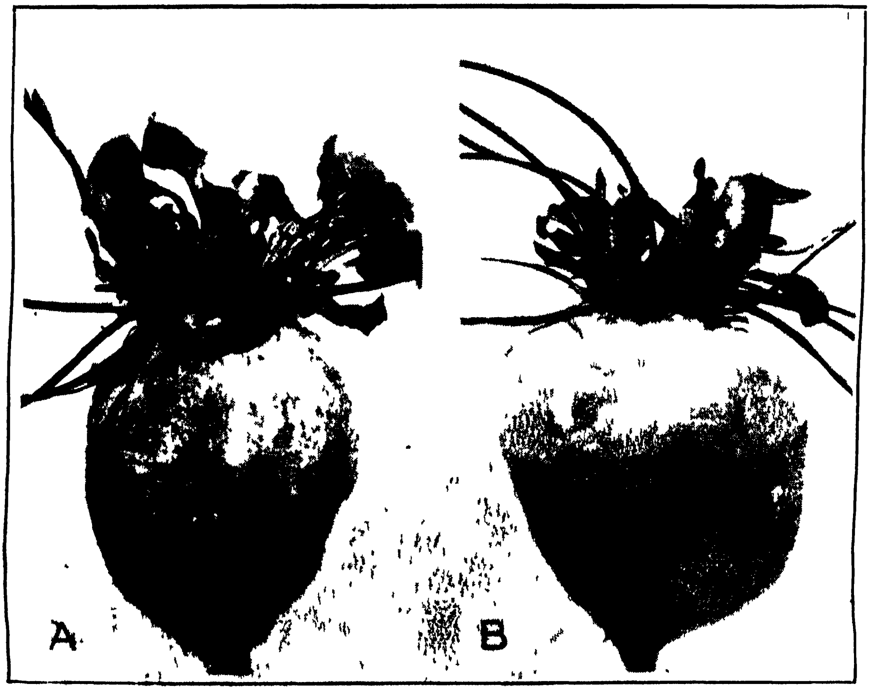

FIG. 2. A. Surface cankers on garden beet that formed following the development of internal black spot near the periphery. *B. A nearly mature beet severely* affected with internal black spot but, in contrast to the one shown in A, without any evidence of surface cankers. In this case the necrosis was confined to tissues removed some distance from the surface.

surprising to find that the superficial cankers often show evidence of having been corked out by the underlying cells. Presence of fungus mycelium on the surface of cankers and within the underlying tissue of peripheral lesions is common, giving the appearance of decay in which parasitic agents have been a major causal factor. Although it is not impossible that secondary organisms play a part in influencing the extent and final configuration of the cankers, a careful study of many specimens and of progressive formation of the lesions leads the writer to conclude that the initial causal factors are identical with those associated with the necrotic areas deeply imbedded in the fleshy root. In figure 2, A, is an example of a large canker which has developed from necrosis near the periphery. Upon cross-sectioning this root it was found that the most pronounced development of internal black spot was in the inter-xylem zones closest to the surface. In figure 2, B, is a root taken from the same field on the surface of which no signs of canker occur. When this root was cross-sectioned quite as large a proportion of it as of the root in figure 2, A, had become necrotic, but the breakdown had occurred at points sufficiently removed from the surface to prevent development of cankers.

Top symptoms are quite characteristic. They are usually correlated with internal necrosis but not always. Sometimes pronounced top symptoms precede root necrosis. As a rule they appear after midseason. The older leaves are normal in appearance while the younger leaves at the center of the crown are the ones which show the signs of the disease. In the first stages the symptoms are malformation and a greater concentration of the red anthocyanin color in the leaf lamina than in normal leaves. The former consists

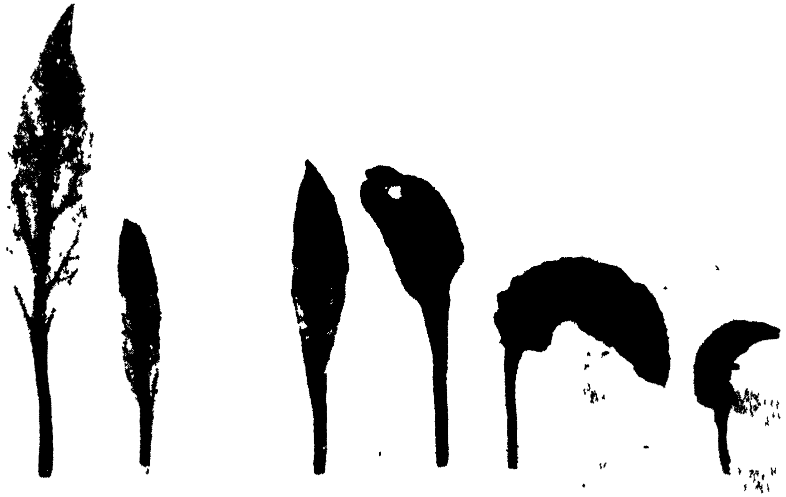

FIG. 3. Early symptoms of internal black spot on the foliage. Note leaf malformation and unilateral development. Normal leaf at the left.

of stunting of the leaves and, in the course of the retarded development, a tendency toward greater length in proportion to breadth. Unilateral development due to greater retardation of expansion of the lamen on one side of the midrib than on the other is often found while in the dwarfed tissue there is a greater concentration of red coloring matter. A slight downward roll of the leaf margins sometimes accompanies the malformation. Certain of the early top symptoms are shown in figure 3.

At certain stages these early symptoms are very striking and through them the half-grown beets with internal necrosis can often be distinguished. The subsequent top symptoms depend somewhat on the growing conditions that prevail. The malformed leaves tend to become necrotic and die early. If the disease has set in while the plant is relatively immature, new leaves, greater than normal in number and showing early signs of red color and malformation may be formed. This type of response is shown in figure 2, A and B. In figure 4 is a comparison of a normal healthy beet and a severely

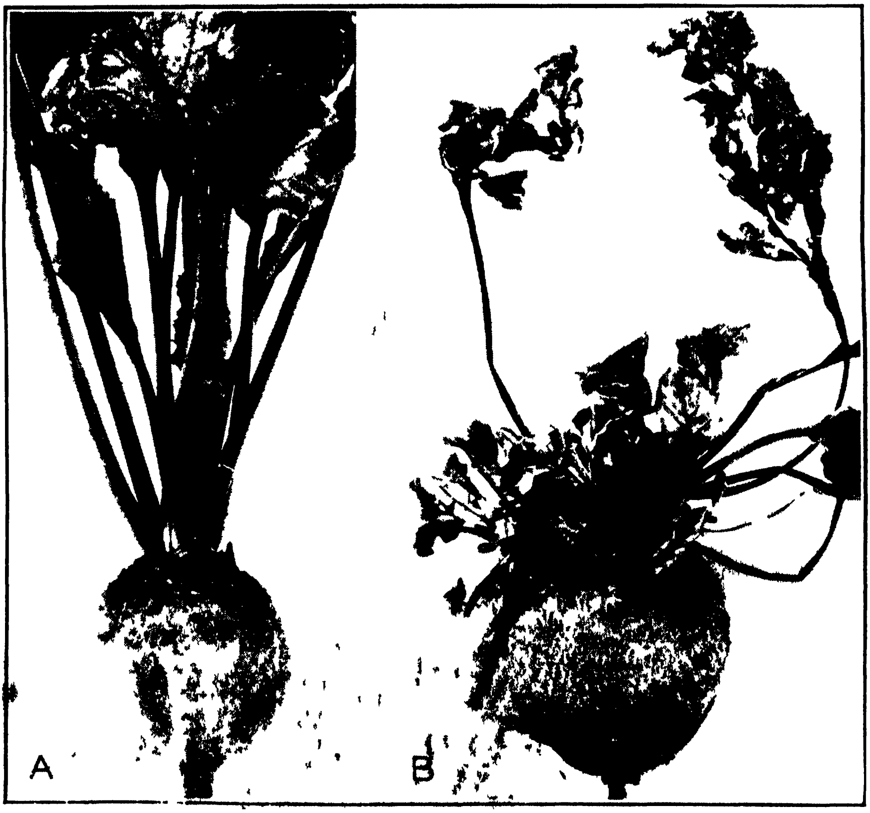

FIG. 4. A. Healthy beet approaching maturity. B. Diseased beet in which the prolonged effect of internal black spot on the foliage is shown, from the same field as that in A. The earliest outer leaves apparently grew to normal size. The inner leaves were successively malformed as were those in figure 3 and died early, resulting eventually in a rosette of dead leaves. Late in the season the dormant buds at bases of some of the dead leaves were stimulated and a few leaves were thus formed throughout the crown.

diseased one both of the same age and grown in the same field. The healthy beet is reduced in magnification in greater proportion than the healthy beet. In the former the leaves fell off successively as they became senescent, leaving scars on the crown, while the active leaves near the center are normal in size and number. In the diseased beet a few outer senescent leaves remain and appear to have grown to about normal size. The remainder of the crown, however, consists of leaves that gained a length of 1 or 2 inches and then became successively necrotic, forming a rosette of short dead leaves. Following this, as the beet would normally approach maturity, there commonly results, as in this case, proliferation of dormant buds at the bases of the dead leaves and the resultant production of new leaves promiscuously throughout the crown. Such leaves usually take on abnormal symptoms similar to those which have preceded.

It may be seen that the top symptoms of internal black spot of garden beet have some points of similarity to the heart and dry-rot sugar beet, which was ascribed to boron deficiency by Brandenburg[1] in 1931. It should be pointed out here, however, that, although the necrotic rosette of the crown is the outstanding feature of sugar-beet heart rot and root necrosis is relatively inconspicuous, approximately the reverse is the case with garden beet where root necrosis is the conspicuous and severe manifestation, while extreme heart rot of the crown is relatively uncommon.

CAUSAL AGENCIES

The possibilities of an organic pathogen were first explored. Over a hundred roots of typically diseased roots were examined. After disinfection of the surface, bits of tissue were removed aseptically from necrotic areas to potato-dextrose agar plates. These fragments remained consistently sterile except when they were obtained from areas which were close to peripheral cankers. In those instances a number of distinct fungi and bacteria were recovered. Inasmuch as no organic growth occurred except where surface contamination was obviously probable, the possibility of a culturable causal pathogen was set aside.

The similarity in many respects to heart rot of sugar beet led to exploration of the possibility of boron deficiency as a causal entity. The experience of beet canners seemed to associate the greatest severity of the disease with seasons of sub-normal precipitation and in high, dry parts of fields as compared with low, moister portions. Numerous exceptions to these correlations, however, were found.

In the spring of 1937 several beet fields were selected at random, in areas where the disease had formerly occurred, extending from Fond du Lac County at the lower end of Lake Winnebago to Kenosha County in southeastern Wisconsin. Boron was applied in the form of commercial borax when the plants were in the second-leaf stage. It was usually spread broadcast between the rows and incorporated in the upper inch of the soil by a hand cultivator. In one field (Brown farm, Fond du Lac County) it was applied in a furrow at one side of the row about 2 inches away from the plants and about 2 inches below the surface. In none of the fields did any injury to the crop result from the application of borax at the rate of 10, 20, and 30 pounds per acre. In fields in which the surface was undulate the treated plots were chosen so as to run from the top of a knoll to a lower level in the field.

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[1] Brandenburg, E. Die Herz- und Trockenfäule der Rüben als Bormangel-Erscheinung. Phytopath. Zeitschr. 3: 499–517. 1931.

Before treatments were applied, representative samples of the upper six inches of soil in each field were taken and each was tested for reaction, available phosphorus, and available potash. In each field a single plot of four rows, approximately 175 feet in length, was treated with each level of borax (10, 20, and 30 pounds per acre). Four-row untreated plots were left at the borders and between the treated plots. All fields had been planted with Detroit Dark Red variety.

Representative samples were removed from the two center rows of each plot when the plants had reached the canning stage. In the Brown farm field a second series of two samples was taken three weeks later. When a high and low portion of the field was being studied samples were secured separately from each. The beets in the collected samples were each cut into slices about $\frac{1}{4}$ inch thick and examined for the presence of internal black spot. The results of this study, together with the soil analyses from each field, are given in table 1.

The soils from fields in Kenosha and Fond du Lac Counties were acid while the Ozaukee County fields studied were alkaline. It may be seen, however, that blackening occurred in all cases. When high and low areas in fields of two nearby farms in Kenosha County are compared the incidence of the disease was decidedly greater in the high area in one field (Lichter farm) and in the low area in the other (Bishop farm). All fields had a fairly good supply of available potash; some were very low in available phosphorus while in none was it adequate. Nevertheless, when the areas with high disease incidence in both the Lichter and Bishop farms are compared with the areas of low disease incidence, no correlation with the amount of available phosphorus is found. In the Brownsville field there was a definite increase in internal black spot in the second harvest, illustrating the observation commonly reported by canners that the disease usually increases progressively after it once appears.

There was a definite tendency toward a reduction in the incidence of internal black spot in the trials on the Brown farm in Fond du Lac County, on the Pepkorn farm in Ozaukee County, and on the Lichter and Bishop farms in Kenosha County. When the results from the respective treated plots are compared with adjacent nontreated plots there was usually a gradual decline in disease with increase in rate of application. In no case, however, was it completely eliminated. In two of the fields (Leuder farm, and Christensen farm), one on alkaline soil, the other on quite acid soil,

TABLE 1.—*Occurrence of internal black spot of Detroit Dark Red beets on nontreated plots and on plots treated with various amounts of borax when plants were in the second-leaf stage; Fond du Lac, Ozaukee, and Kenosha Counties, Wisconsin, 1937*

Treatments and soil analyses	Brownsville, Fond du Lac County		Cedarburg, Ozaukee County			Somers, Kenosha County					
	Brown farm		Leuder farm	Pepkorn farm		Lichter farm		Christensen farm		Bishop farm	
	Pulled Sept. 10	Pulled Oct. 1		High area	Low area	High area	Low area	High area	Low area	High area	Low area
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Nontreated	37.6	53.9	29.8	42.9	50.9	51.3	12.2	23.4	12.5	7.6	35.8
10 lbs. borax per A.	24.3	44.0	26.9	25.9	17.1	38.3	9.3	12.0	16.7	6.0	26.8
Nontreated	28.2	52.1	31.5	26.0	28.3	31.8	4.6	4.4	12.9	15.0	47.6
20 lbs. borax per A.	11.2	30.3	26.8	12.5	14.7	13.1	4.8	3.0	27.9	3.7	19.5
Nontreated	15.2	44.4	24.1	28.8	45.7	33.7	10.1	5.9	10.3	2.3	38.0
30 lbs. borax per A.	8.1	18.8	59.4	18.8	12.9	20.7	3.2	7.3	14.3	7.0	23.9
Nontreated	7.6	52.0	22.9	14.5	14.1	36.8	8.5	2.0	31.5		31.3
Soil analysis of composite sample of untreated plots:											
Reaction (pH)	5.0		7.5	7.5		5.5	5.5	5.5		5.0	6.0
Available phosphorus (lbs. per A.)	10		20	20		60	50	60		60	65
Available potash (lbs. per A.)	175		200	200		350	225	200		225	225

there did not appear to be any consistent benefit in the reduction of internal black spot by the application of borax.

DISCUSSION AND SUMMARY

The internal black spot of garden beets has become rapidly an extremely important disease in the canning industry. The symptoms are quite distinctive on roots and tops and they have much in common with those associated with the boron-deficiency disease in sugar beets, known for many years as heart rot. The results presented herewith indicate that there is no pathogenic parasite involved as a fundamental causal entity. A survey of its occurrence in Wisconsin shows it to be prevalent under a variety of soil conditions. During the latter part of the 1937 season a rather extensive survey of fields in which the disease occurred showed that a majority of these were on alkaline soil. The limited trials with the application of borax in 1937 indicate some beneficial effects. In view of the fact that trials in New York State in the same year by Raleigh and Raymond[2] also greatly reduced the disease in one field in which borax was applied, it would appear that further study of the relation of the deficiency of minor elements in relation to its development is justifiable. These results are offered as a report of progress while further experimental work is under way.

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THE TAXONOMY AND NOMENCLATURE OF THE PHYTOPATHOGENIC BACTERIA

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In a young and growing science like Bacteriology there is a constant need for revision of taxonomic concepts. Since 1923 there have been 4 revised editions of Bergey's "Manual of Determinative Bacteriology," and a fifth edition is now in the press. The committee of bacteriologists which have undertaken the revision of the new edition have laid down certain general principles, but they have been free to call upon a number of persons for advice concerning certain groups. The writer has rendered such assistance as he has been able in connection with the bacterial plant pathogens, and a step has been taken towards their reclassification. It is proposed here to discuss the criteria that have been taken into consideration with new arrangements of these organisms. This step in the forthcoming edition is not final and has not gone so far as to make any changes in generic names. It consists, mainly, of an attempt to show the natural relationships of the genus *Erwinia* and of the various groups of the genus *Phytomonas* in the order Eubacteriales.

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[2] Raleigh, G. J. and C. B. Raymond. A preliminary note on the control of internal breakdown in table beets by the use of boron. Proc. Amer. Soc. Hort. Sci. (1937) 35: 526–529. 1938.

Eventually new generic names must be proposed for the natural groupings; but to establish them on worn-out generic concepts and ignore the accumulating data on these species would not be progressing from our old classifications. On the other hand, the hurried acceptance of new concepts might be equally ineffectual. Merely to give *Phytomonas* a new name because of the resolution passed by the Second International Congress of Microbiology (9), which states that "generic homonyms are not permitted in the group Protista" would be complying with this recommendation, which perhaps is not meant to be retroactive, but would not be improving the classification of these organisms. It would be assuming that the genus is based on sound characters.

The genus *Phytomonas* has been discussed before (3), possibly not too well, in the light of our present knowledge, but sufficiently to show that it contains a heterogenous group of bacterial plant pathogens. Such being the case, the present generic characters of possession and position of flagella, used for phytopathogenic bacteria, do not appear to be natural ones. These characters are not without significance, but to use them alone to limit genera has magnified their value out of all proportion. Probably the use of any one single character in like manner would have led to similar confusion. The use of flagella possession and position in correlations with other characters, on the other hand, is very helpful at times. Among the bacterial pathogens those possessing peritrichic flagella show a close relationship while those possessing polar flagella show less of a relationship. Other characters must be used here, and the present trend is along physiological lines.

The absence of motility among the phytopathogenic bacteria is not generic and at times it scarcely appears to be a specific character. *Phytomonas dissolvens* Rosen shows little relationship to *Phytomonas insidiosa* (McCulloch) Bergey *et al.* or *Phytomonas pseudotsugae* Hansen & Smith, yet all are non-motile. The first-named species is, in all characters except motility, closely related to *Erwinia carotovora* (Jones) Holland, the second possibly to the genus *Chromobacterium* and the last to *Rhizobium*. These last two genera, however, belong in the same family. Adams and Pugsley (1) found a non-motile strain of *Phytomonas flaccumfaciens* (Hedges) Bergey *et al.*, but it would not seem wise to remove the species to another genus on that account. This case is not uncommon, since non-motile strains have been reported among many bacterial species normally motile. Our best authorities appear to be somewhat in doubt as to whether *Phytomonas tumefaciens* (Smith and Townsend) Bergey *et al.* is motile or not. Its generic name therefore is somewhat doubtful in certain classifications. For many years the character of motility and especially its absence has been used less and less in favor of physiological characters.

Recently Dr. R. S. Breed[1] pointed out that, even if the non-motile species did form a good group, the name *Aplanobacter* probably could not be used for them since E. F. Smith (13) designated *Bacillus anthracis* Cohn, a non-motile, spore-former, as type species. While Smith did not bar non-spore-formers from the genus, he does state that they possibly do not belong there. All aerobic spore-formers, motile or not, now are placed in the genus *Bacillus.*

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[1] Letter.

Among the motile species in *Phytomonas,* which have one to several polar flagella, the green flourescent *Phytomonas syringae* (van Hall) Bergey *et al.* scarcely can be considered closely related to the gram-positive *Phytomonas flaccumfaciens. Phytomonas campestris* (Pammel) Bergey *et al.* is not closely related to either of these. Those who have worked with the three species see many differences, and each of these species represents a distinct physiological group.

Miss Elliott (6) in a recent note appears to accept the genus *Erwinia* but throws out the genus *Phytomonas* on the resolution of the London Congress referred to above; and states that "For the present at least there are two alternatives for the plant pathologists," that is, Migula's classification or E. F. Smith's classification. She forgets that of Lehmann and Neumann recently used by Burgwitz (2) in his book on phytopathogenic bacteria. I think that *Phytomonas* as it stands does not comprise a natural group and I possibly agree with her in the undesirability of the name. We cannot, however, go back to Migula's or to E. F. Smith's classification or to Lehmann's and Neumann's. Our accumulating knowledge of the bacteria, especially of the rod forms in which the plant pathologists are interested, show that these systems which are based entirely on morphology place the bacteria in unnatural groups. Such systems have become antiquated. For the present and for some time to come, nevertheless, various plant pathologists will go on using these classifications, as in the past, and sometimes unknowingly two or three systems in the same article. Possibly we cannot help this, but we can work to secure a system of classification in the future which will be more or less acceptable to all. In doing this one must remember that the phytopathogenic bacteria are only a small group of the Schizomycetes, and only a systematist in bacteriology can place them intelligently. The plant pathologist working with the organisms can obtain information concerning them, arrange them into groups on the information, and present them to the taxonomist in bacteriology. Here they should be made into genera or incorporated into genera already existing.

At present the Bergey Manual classification is the only one that is flexible, and is developing. It is based on a combination of morphology and physiology, stressing what appears to be important in each group. New editions come out from time to time correcting the mistakes of the last and endeavoring to show natural relationships in the bacteria. It is also the only publication containing a description of all known bacteria. The system is being followed more and more, and with the editors endeavoring to abide by the international rules of nomenclature it possibly will become universal. Recently Paul Hauduroy, G. Ehringer, Ach. Urbain, G. Guillot, and J. Magrou in their "Dictionnaire de Bactéries Pathogenes," have adopted the classification (8).

As stated above, in the 5th edition of Bergey's Manual a step has been made in the reclassification of the bacterial plant pathogens. Certain of the objectionable features of classification of this group of pathogens found in the earlier editions are being remedied. It is no longer considered that the plant pathogens form a distinct group of their own, and the concept that plant pathogenicity is a generic character is being weakened. All the pathogens described as spore-formers have been removed from *Erwinia* and *Phytomonas* and placed in the genus *Bacillus.* There are some twenty odd of these and it is likely that they all are saprophytes or have been described from mixed cultures. The majority were described 20 years ago or more, and none appear to have been investigated carefully since their first description.

The genera *Erwinia* and *Phytomonas* have been placed in separate families, since the species in one genus show no close physiological relationship to the species in the other. The tribe *Erwinieae* with its single genus *Erwinia* is placed in the family *Enterobacteriaceae,* a new family comprising *Escherichia, Aerobacter,* etc., which include many intestinal and some soil bacteria. It has been recognized for some time that *Erwinia carotovora* shows some relationships to the *Escherichia-Aerobacter* group. Frequently in the past, plant pathologists have held *Escherichia coli* (Migula) Castellani and Chalmers to be the cause of various rots of plants. In many instances the description of the plant pathogenic *Escherichia coli* leaves one in doubt as to whether the investigator had a strain of *Erwina carotovora* or something that resembled a species of *Aerobacter.* Certain of the species of the latter genus are ubiquitous soil bacteria and no doubt could be found associated with a root rot if not actually the cause of it. The similarities and differences of the species of *Erwinia* to those of *Aerobacter* and related genera must be investigated before it will be possible to determine whether the genus *Erwinia* should stand or the species be absorbed in one or several of the closely related genera. F. D. Chester has prepared the genus *Erwinia* for the 5th edition of Bergey, and I have not studied this group sufficiently to make comments on his arrangements. However, 3 species usually placed in the genus *Phytomonas* are now under *Erwinia* and some explanation should be made. Harding and Morse (7), and later Paine (10) presented evidence that indicated that the organism Potter (11) described as *Bacterium destructans* was either *Erwinia carotovora* or a related species and we have followed these investigators. Dowson (5) showed that the cause of water mark of willow, *Bacterium salicis* Day, is an *Erwinia,* similar to *Erwinia amylovora.* And *Phytomonas dissolvens* Rosen (12) from the descriptions can only be a non-motile strain of *Erwinia carotovora.* Rosen states that it is very similar to *Escherichia coli* and Stanley and Orton (15) state that "It shows striking similarity to *Bacillus carotovorus.*"

The genus *Phytomonas* as it now stands contains by far the larger number of the bacterial plant pathogens. In the 5th edition of Bergey's Manual I have given a description of 149 species and varieties. Furthermore, there are 40 synonyms and new names listed; and 33 specific and varietal names placed in an appendix. No doubt a few species in the literature have been overlooked. It appears likely that some of these recognized species listed are in reality synonyms, but such can be proved only on investigation. Species described since August, 1937, have not been included since the manuscript was completed shortly after that date.

In the key, the species of the genus have been divided into three groups, which are more or less those arrived at in 1930 (3), and are based mainly on the physiology of the organisms. The largest group is built around the species that produce a blue or yellow green fluorescent pigment in certain media. This single character does not limit it, however. Clara (4) showed that the green fluorescent species he worked with were a very closely related group. In literature many species are to be found with these same characteristics but lacking the ability to produce this pigment or the pigment has not been observed or been reported. Wilson (16) showed that strains of *Phytomonas cerasi* (Griffin) Bergey *et al.* occurred without the pigment. Such pathogens therefore should be included in the group. We have characterized this group as composed of medium-size, gram-negative rods, frequently curved, white to cream, usually motile with one but more often several polar flagella. The species grow well in media containing inorganic nitrogen as the only nitrogen supply; produce acid from the monosaccharids, and in certain cases from sucrose. H_2S is not produced or a feeble production has been reported. Action in milk is alkaline and if the medium is cleared (by a number of species) it is a simple clearing due to an alkaline reaction. Fluorescin is produced readily by many species in a liquid synthetic medium where asparagin is used both as the carbon and the nitrogen source. Here a very good growth is obtained. The pathogens produce a necrosis in plants. This group is related to *Pseudomonas* of Bergey but this latter genus is limited entirely on pigment production, and without certain modifications the two genera scarcely can be placed together under a single name.

The next largest group is built around *Phytomonas campestris,* which is the type species for the genus *Phytomonas.* E. F. Smith (14) recognized this group, but as far as I am aware never referred to it in a taxonomic paper. The pathogens are medium size, gram-negative rods, mostly yellow, motile usually by means of a single polar flagellum. Their growth on potato-dextrose agar is very characteristic, being profuse and gummy. The organisms grow well in synthetic media with inorganic nitrogen as the sole source of this element. Acid is produced from mono-saccharids and from disaccharids. Some species hydrolyze starch. H_2S is produced. Growth in milk results in an alkaline medium with a soft curd-like mass in the bottom of the tube, which more often peptonizes with the production of crystals. Most species liquefy gelatin. This group does not utilize asparagin as a source of carbon and nitrogen, as the green fluorescent forms do. The pathogens as a rule produce a necrosis in plants.

The third group is composed of fewer species and they are not so closely related as the species in the two previous groups, although they show certain characteristics in common. They are small rods, yellow or white, gram-positive or negative, non-motile or motile with polar flagella, and they cannot be separated into natural groups on any of these characters. Growth in media is very slow, especially with recently isolated strains. Most species do not grow with inorganic nitrogen. H_2S production none or weak. Sugars appear to be utilized, but not with much acid production. Action on milk none or acid. Gelatin not as a rule liquefied. Pathogens produce hypertropies and wilts. Three species, which produce rots, have been placed in this group but they are rather doubtful pathogens. They are not well described and each has been reported but once.

In this third group, the pathogens that produce galls are without doubt closely related to organisms in the genus *Rhizobium* and the soil organism *Bacterium radiobacter.* Those that produce wilts show relationships to the gall producers, and it might be pointed out that *Phytomonas insidiosa* (McCulloch) Bergey *et al.* produces a purplish pigment and might belong to the genus *Chromobacterium*, which is in the same family with *Rhizobium.* I have placed *Phyt. fasciens* Tilford with the gall producers but on further study it might be found to belong near *Phyt. beticola* (Smith, Brown, and Townsend) Bergey *et al.*

Certain species, which have been described very poorly, have caused some trouble in placing them in their proper group. Whenever a complete description is given of a pathogen, the species fits very readily into one of the three groups of *Phytomonas* or into the genus *Erwinia.* In some instances, however, one may find a discrepancy between a species and its group; a gram-positive organism in a gram-negative group, but certain laboratories seem to turn out more than their share of gram-positive species. In other cases the utilization or absence of utilization of carbon-compounds is not always logical. Too often we must rely on a description of an organism that was reported a number of years ago and has never been reinvestigated.

For the extreme cases of these discrepancies listed above an appendix has been used. In it those species have been placed that are inadequately described, but which might on further investigation be found to belong to one group of the genus *Phytomonas.* These species are not entirely discarded, and their names are listed for further reference. Too often an investigator publishes a name with no description, *nomen nudum* or a slight, meager description. He evidently hopes after fastening h , name to the species to give later a full description of the pathogen, which sometimes he does and at other times he does not. In either case confusion arises in the taxonomy of the species. A well described species with some other name attached is more helpful than a new species nude or scantily clothed.

The genus *Phytomonas* has been placed next to *Pseudomonas* in the family Spirillaceae. The relationship of the green fluorescent plant pathogens to this genus is fully understood, and for those wedded to the morphology idea it might be pointed out that of these green fluorescent pathogens many are curved rods and not unlike *Vibrios.* The *Phytomonas campestris* group possibly could be placed in this family but those pathogens in the third group appear to belong elsewhere.

The key devised for the species in the genus *Phytomonas* is not very workable in the lower brackets. This is unavoidable and is due to the fact that many of the species can be separated only on their pathogenicity to certain plants or groups of plants. Such a condition would lead one to believe that culturally many are identical, and this is true as far as we are able to determine from the published descriptions. Although some of these descriptions, as stated previously, are inadequate, many species have been described in detail using our present bacteriological methods and show no cultural or biochemical differences. Further tests not already in use might be devised which would bring out differences; or they might not. Search for such tests appears to be a worthy project, however.

Furthermore, the fact that many of these pathogens show no differences in cultures might lead one to believe that many are synonyms. In recent years considerable work has been conducted on cross inoculations with similar appearing pathogens with the outcome that there are approximately 40 species listed as synonyms. Further work, no doubt, should be done along this line, and with the description of a new species, cross inoculations should be made on the hosts of similar appearing pathogens. In making synonyms for the Bergey Manual I probably have been a little lax in certain cases. *Phytomonas spongiosa* (Aderhold and Ruhland) Magrou, judging from its description and from the host it attacks, is probably a synonym of *Phytomonas cerasi,* but we have no definite proof as yet. Also Wilson (16) is inclined to believe that *Phyt. cerasi* is a synonym of *Phyt. syringae* and I must admit that, at present, indications point in that direction. Since he has failed to state this definitely, I have considered them still two species.

On the other hand there are pathogens fully investigated that have not been differentiated in culture, but that limit their pathogenicity to a distinct host. Wernham[2] worked with 18 species closely related to *Phytomonas campestris* and found none that cross infect. These members of the *Phyt. campestris* group as a rule are limited to a single host species or to a few species in a genus. The green fluorescent pathogens appear to be the ones with a wide host range.

This phenomenon of several species appearing similar in culture and differing in pathogenicity has brought up a problem in nomenclature. In certain instances many pathogens have been given specific names and their similarity in culture to another pathogen has not been noted. In other instances the similarity has been perceived and a varietal name given. Since no definite rule has been followed many of the species are more closely related to each other perhaps than the varieties are to the species. When one looks over the species and varieties in the genus one does not get a true picture of the relationships. I have erred in the past in giving varietal names, and I am inclined to think that specific names are less confusing.

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[2] Wernham, C. C. A comparative study of some yellow proteolytic bacterial plant pathogens. (Thesis, Cornell University (unpublished).) 1936.

In examining the relationships of the bacterial plant pathogens to other genera of the *Eubacteriales,* one of the most interesting points to raise is the possible origin of these forms. The origin is no doubt where one should look for it—among those ubiquitous soil bacteria. These bacteria are in close contact with the plant roots and also they are blown about over the aerial parts of the plants. Pathogenic races have a chance to develop. The species in *Erwinia* apparently arise from the soil forms of *Aerobacter* or its related genera. The green fluorescent pathogens come from *Pseudomonas fluorescens* Migula, which is ever present in soil and water. The gall producers arise from *Bacterium radiobacter* and its kin, as do also the species in the genus *Rhizobium.* If we knew more about the gram-negative non-spore-formers of the soil we might also find the origin of the yellow *Phytomonas campestris* group. There are common yellow saprophytes found everywhere on the surface of plants that frequently are referred to as *Bacterium herbicola aureum,* but this can scarcely be the progenitor of the group, since the ones I have examined have resembled organisms usually placed in the genus *Aerobacter,* motile with peritrichic flagella and sometimes producing gas. Perhaps all of our bacterial forms have arisen from the soil bacteria, but the evolution of the plant pathogenic forms has not been of sufficient duration to get far away. Why we find none or few spore-formers is a problem only to speculate about at present.

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PHOMOPSIS TWIG BLIGHT OF BLUEBERRY

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INTRODUCTION

A species of *Phomopsis* has been known as the cause of a serious storage rot of cranberries, *Vaccinium macrocarpon* Ait., since 1916 (3). In 1931 (4) the imperfect and perfect stages of the cranberry *Phomopsis* were described respectively as *Phomopsis vaccinii* and *Diaporthe vacinii.* The pycnidial stage was reported to occur on fruits and the perithecial stage in cultures from fruits and on decayed berries and dead vines. The writer has found *Phomopsis vaccinii* fruiting on overwintered cranberry leaves and has cultured it from buds and from green cranberries in both Massachusetts and New Jersey.

Every spring, since 1934, a similar species of *Phomopsis* has been isolated from blighted new shoots of the cultivated blueberry, *Vaccinium corymbosum* L., collected at or near the State Cranberry Experiment Station, East Wareham, Massachusetts, by the writer or by H. F. Bergman, also of the Bureau of Plant Industry. In 1936 (5) the blueberry twig blight was reported to occur sparingly in the commercial blueberry-growing sections of Massachusetts, New Jersey, and North Carolina. Pycnidia of this fungus also have been found on blueberry leaves and on dead, overwintering twigs. Cultural and inoculation studies reported herein establish the identity of this blueberry *Phomopsis* with *P. vaccinii* in cranberries.

MATERIAL AND METHODS

Cultures of *Phomopsis* were obtained from small sterilized pieces of naturally blighted blueberry shoots and from the pulp of decayed cranberry fruits, both collected near East Wareham, Mass. Twenty-four seedling blueberry plants resulting from crosses of Katharine × Rubel and Stanley × Weymouth, and 2 plants each of the varieties Katharine and Scammell were used as test plants. Preliminary greenhouse experiments indicated that the most favorable temperature for growth of the fungus and germination of spores was 70° to 75° F. Comparable inoculation and cultural studies were made, using isolates from both hosts:

1. Young succulent blueberry shoots were (a) atomizer-sprayed with a suspension of spores in tap water and held under a bell jar for 3 days; (b) inoculated with mycelium. After swabbing the surface of the shoots with 1:1000 mercuric chloride in 70 per cent alcohol, and rinsing with sterile water mycelium was placed in wounds or on the unbroken bark and protected by wrapping in moist cotton and waxed paper.

2. New and second-year woody tissue was (a) inoculated with mycelium with and without wounding; and (b) sprayed with a suspension of spores. These inoculations were made in the manner described under No. 1.

3. Individual leaves were sprayed with a suspension of spores and the plants placed under bell jars for 3 days.

Adequate controls were used in all series of inoculations.

RESULTS

1. (a) Inoculation of Succulent Shoots with Spore Suspensions

In July, 1937, succulent shoots originating near the crown of 4 seedling plants were sprayed with spores and held in a damp chamber at room temperature for 3 days. The *Phomopsis* isolated from blighted blueberry shoots and *Phomopsis vaccinii* from rotten cranberry fruits were each used on 2 plants, and 2 plants were sprayed with water and held as checks. Cultures made from small sterilized pieces of the inoculated plants, 24, 48, and 60 hours afterward, produced *Phomopsis* in all cases after the 48-hour interval. Within 4 days the inoculated shoots were wilted and peppered with minute lesions. They all recovered somewhat, but did not survive the winter, and when examined in February, numerous *Phomopsis* pycnidia were found erumpent through the bark. There was no infection at any time in the control plants.

In March, 1938, succulent shoots were sprayed with a spore suspension of the blueberry isolate and held in a damp chamber 3 days at a temperature of 70° to 76° F. Microscopic examination of spore suspensions collected from twig surfaces disclosed 95 per cent germination within 24 hours. Four days after inoculation 71 per cent of the young growing shoots were blighted at or near the tips (Fig. 1, A). The fungus progressed toward the bases of the shoots at a fairly rapid rate and, after completely killing them, entered and girdled the older main branches of the plants, causing the death of all branches beyond the girdled areas. Within 5 weeks a considerable portion of the plant was dead (Fig. 1, B). The diseased tissue was dark brown or almost black and separated from the healthy light-green bark by a sharp line of demarcation. *Phomopsis* was reisolated from the infected areas and grown in agar cultures, which later produced pycnidia and spores. Further reinoculations from these reisolations resulted in typical twig dieback.

Identical inoculations, using *Phomopsis vaccinii* from rotten cranberry fruit, were made. Microscopic examination of spores in drops taken from the surface of atomized tissue showed only 1 per cent germination after 48

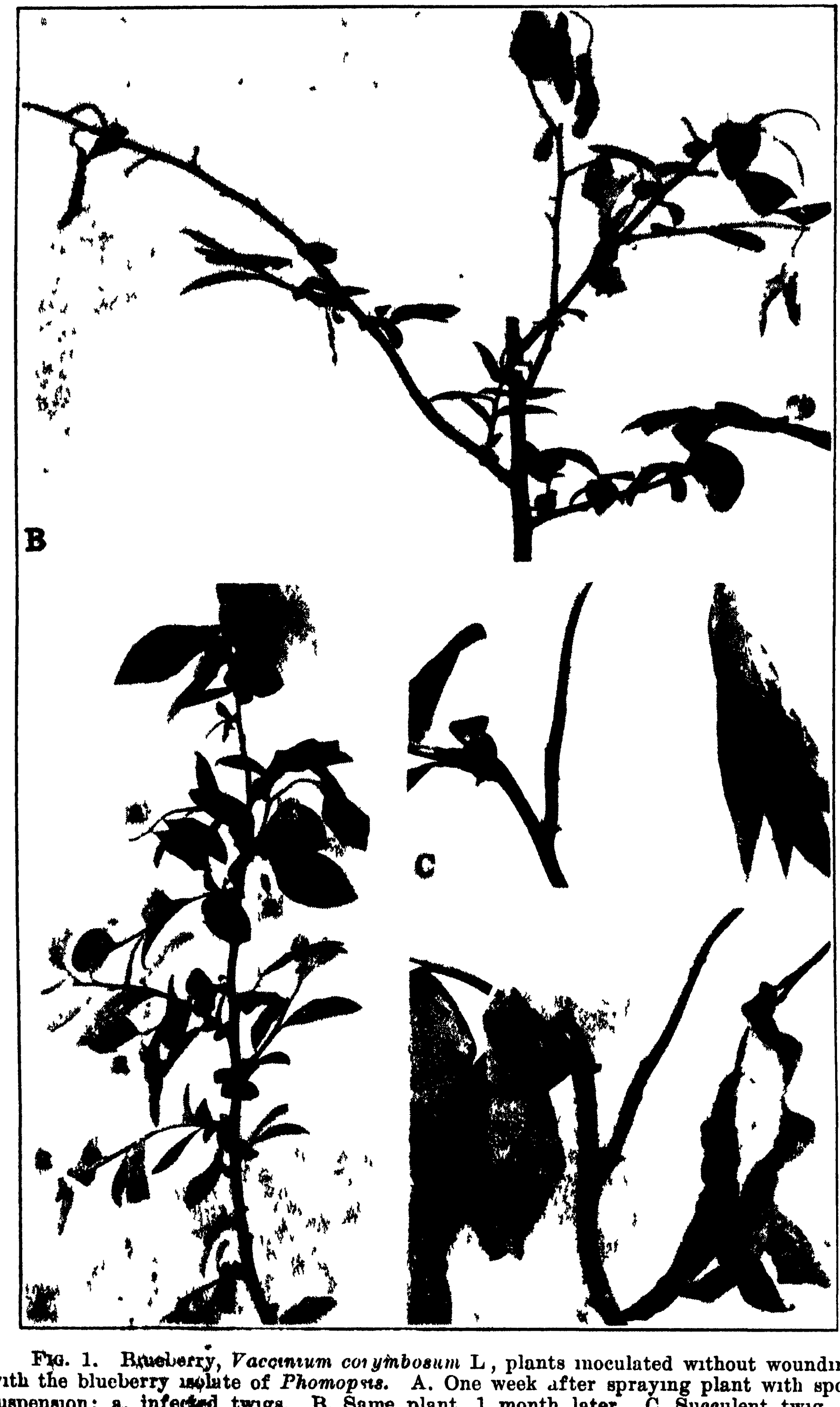

FIG. 1. Blueberry, *Vaccinium corymbosum* L., plants inoculated without wounding, with the blueberry isolate of *Phomopsis*. A. One week after spraying plant with spore suspension: a, infected twigs. B. Same plant, 1 month later. C Succulent twig, 11 days after inoculation. D Girdling of second year wood of same branch 9 days later.

hours. Despite this low percentage of germination, over half the twigs became infected at the tip within a week. The progress of the disease in new tissue and the girdling of the old wood were similar in all essential respects to the inoculations made with *Phomopsis* obtained from naturally infected blueberry twigs.

1. (b) Inoculation of Succulent Shoots with Mycelium, with and without Wounding

To study further the pathogenicity of *Phomopsis* from both hosts, succulent blueberry twigs 4 to 6 inches long were inoculated with and without wounding the tissue. When mycelium of the blueberry strain was applied to the unbroken surface, infection was obtained in 6 of 8 twigs. Upon removing the cotton 2 days after inoculation it was found that the fungus had penetrated the bark, forming blackened lesions 2 to 3 mm. long. Within a week the disease had progressed nearly 2 cm., and in some cases had girdled the twigs (Fig. 1, C and D). *Phomopsis vaccinii* originating from cranberry produced the same symptoms in 4 inoculated twigs.

Mycelium of both the blueberry and cranberry strains of *Phomopsis* was inserted in needle scratches in the bark of 7 and 8 twigs, respectively. The moist cotton coverings were removed in 2 days, in each case disclosing darkened lesions that increased rapidly in size, girdling the twigs in from 7 to 10 days (Fig. 2, A, B, D, E). None of the check twigs showed any evidence of the disease.

2. (a) Woody Tissue Inoculated with Mycelium, with and without Wounding

Phomopsis usually enters at the tip of succulent twigs of the current year and works downward, ultimately girdling the second-year wood. The downward progress of the disease in blueberry twigs is fairly rapid, averaging 5.5 cm. in 2 months. Despite the virulence that the fungus attains when inoculated into succulent tissue, only localized lesions are formed when woody growth is inoculated.

Sixteen wound and 4 surface inoculations were made with the blueberry strain on woody growth of the current year and on second-year growth. Except for one instance in which there was no infection, small rose to burnt-umber (2) calluses developed on the bark, with no material increase in size after the first 10 days (Fig. 2, C). No infection was obtained in the 5 twigs held as checks. Identical results were obtained with comparable inoculations on old twigs with *Phomopsis vaccinii* from cranberry.

2. (b) Spores of Both Strains of *Phomopsis* Sprayed, on Woody Twigs

On the fourth day, after incubation for 3 days in a damp chamber; a spattering of pinpoint-size spots appeared along the cane, increasing in number for 2 weeks. In a few cases the spots coalesced to form tiny lesions in the bark surface, but no permanent establishment of the fungus was obtained in this way.

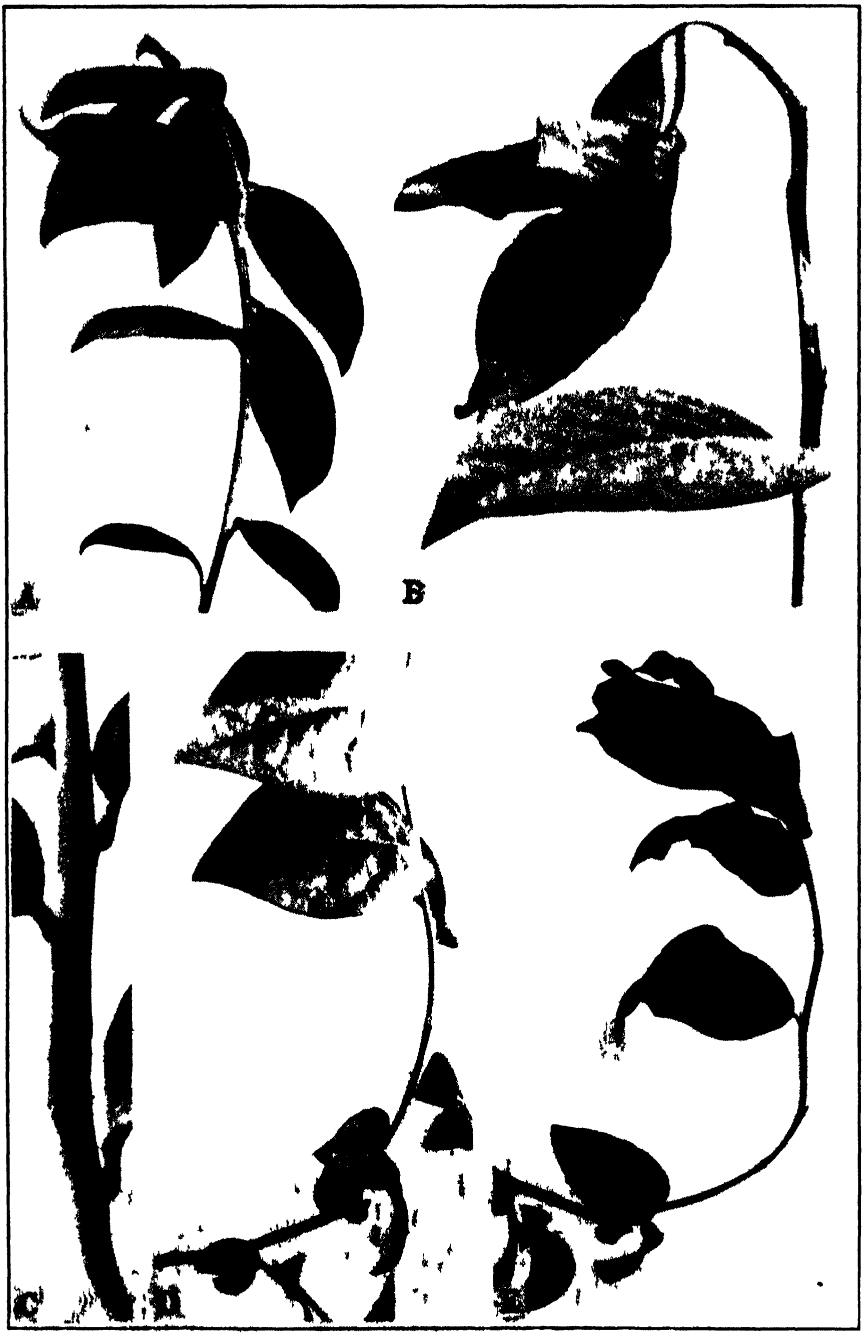

FIG. 2 Blueberry, *Vaccinium corymbosum* L., shoots inoculated by wounding tissue. A and B. Blueberry isolate of *Phomopsis* used, 5 and 12 days after inoculation C. Same isolate, second year wood, 6 weeks after inoculation D and E Isolate of *Phomopsis vaccinii* obtained from cranberry used, 5 and 12 days after inoculation

3. Inoculation of Leaves with Suspension of Spores

When very young leaves were sprayed with a suspension of *Phomopsis* spores, spots rarely developed on them at any time during the season. However, on March 25, 1937, 50 mature blueberry leaves were sprayed with each strain of *Phomopsis* and held under a bell jar for 3 days. There was no evidence of infection by June 21, when the plants were transferred from the greenhouse to the partial shade of a lath house. A week later 21 small reddish spots appeared on 13 leaves of the plant inoculated with the blueberry *Phomopsis*, and 12 spots on 10 leaves of the plant inoculated with *P. vaccinii.* Within another week the number of spots more than doubled. The most probable explanation of this is that infection had taken place in March, but the fungus remained in a more or less dormant state in the tissue until more favorable conditions (lower temperatures) permitted it to resume growth. The spots increased in size to approximately 10 mm. and produced pycnidia from which reisolations were made.

No significant differences were observed between the 2 isolates of *Phomopsis* when grown on Thaxter, beef, strawberry, cornmeal, and potato-dextrose agars at 10°, 18°, and 25° C., respectively. Pycnidia were produced in culture by both strains, and the pycnospores were similar in size and shape in each isolate, having a range of 6–11 μ by 2–5 μ. Scolecospores were produced in abundance by both isolates on some media.

This *Phomopsis* causing a twig blight should not be confused with one reported recently by Brown (1), which induces galls on the crown and along the stems of blueberry plants Not only is there a difference in host reaction between *Phomopsis vaccinii* and the gall-producing *Phomopsis,* but comparative cultural studies showed a decided difference in both growth rate and color.

SUMMARY AND CONCLUSIONS

Inoculation experiments reported in this paper demonstrate the pathogenicity of the *Phomopsis* isolated from blueberry twigs and its apparent identity with *Phomopsis vaccinii* from cranberry. Young succulent blueberry shoots were blighted by both strains of *Phomopsis,* regardless of the methods used for inoculation. The fungus enters near the tip of succulent shoots and progresses toward the base, ultimately girdling the old branches at their junction with infected new tissue, thus killing the part of the plant above the girdle. The disease may continue downward in the woody tissue at a fairly rapid rate, averaging 5.5 cm. in 2 months. However, if woody tissue is directly inoculated, only localized lesions are formed. Under favorable conditions *Phomopsis* may produce spots on leaves on which pycnidia develop.

No differences in the susceptibility to either strain of *Phomopsis* were detected in plants of the two seedling selections, or the varieties Katharine and Scammell used in these inoculations. The *Phomopsis* twig-blight disease is of minor importance, but, so far as has been observed, most of the standard varieties are susceptible to infection. Pycnidia have been found occasionally on dead twigs of Pioneer, Cabot, Wareham, Rubel, Rubel × Chatsworth, and Rubel × Haines collected in Massachusetts, and on Rancocas and Rubel collected in New Jersey.

Identical results were obtained in inoculation experiments when both strains of the fungus were used on blueberry plants. Both forms also showed similar reactions when grown on artificial media. The species of *Phomopsis* causing a blueberry twig-blight is considered identical with *Phomopsis vaccinii* Shear, Stevens and Bain (4), cause of a decay of cranberry fruits.

DIVISION OF FRUIT AND VEGETABLE CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY.

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STUDIES ON FIRE-BLIGHT OOZE

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INTRODUCTION

The nature of the exudate or ooze so characteristic of fire blight, caused by *Erwinia amylovora* (Bur.) Winslow *et al.*, has apparently received little consideration by other investigators. The work of Pierstorff (17), Ark (1), Rosen (20) and some unpublished data detailed in this paper show that the bacteria in dried ooze stored at room temperature as long as 2 years or more may still retain their viability and pathogenicity, whereas those produced on culture media and dried cannot ordinarily be kept as long as one month. The present study was undertaken in an effort to obtain a better understanding of the ooze matrix, which gives such protective properties to the bacteria.

MATERIALS AND METHODS

For the longevity studies, dried beads of fire-blight exudate were collected principally from the naturally infected fruits of Northern Spy apple and several varieties of pear during the growing seasons of 1932, 1933, and 1934. Immediately after collection the dried ooze was placed in vials and

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[1] The writer wishes to acknowledge with gratitude the assistance of Dr. S. F. Howell in repeating and verifying the chemical tests and the courtesy of Prof. H. H. Whetzel in critically reading the manuscript.

stored at laboratory temperatures. Monthly, thereafter, tests for viability and pathogenicity were made as long as the bacteria remained alive.

Because it was impracticable to obtain the ooze in the orchard in sufficient quantity or purity, its production was undertaken in the laboratory. Green pear fruits were selected for inoculation on the assumption that the ooze produced therefrom is identical with that produced on other parts of susceptible plants. It was found after several trials that inoculated slices of green Kieffer pear fruits, handled under approximately aseptic conditions, in moist chambers produced good yields of ooze. Pear fruits picked about August 1, when 1¼ to 1½ inches in diameter, bedded in oiled paper, and stored in tin cans[2] at 32° F., kept for a year or longer.[3] By making tangential cuts, 4 to 5 slices were obtained from each fruit. As each slice was removed it was placed, cut side up, on sterile paper in the moist chamber. Inoculation consisted in the transfer of the inoculum with a 5 mm. loop to the center of the slice. Within 7 to 12 days thereafter (Fig. 1), from 5 to 12 drops of ooze were collected with a pipette from each slice Thus, from each fruit, 1 cc. or more of ooze usually was obtained. Over 50 cc. of such ooze was prepared in each of the 3 series of experiments conducted.

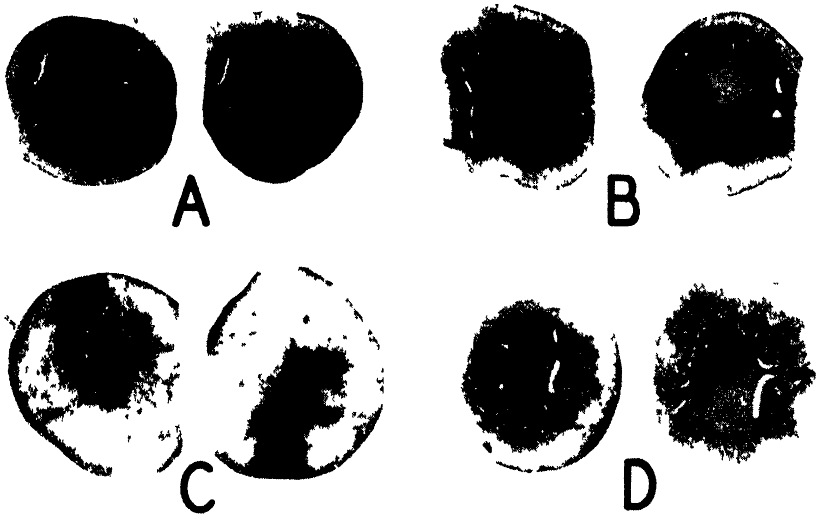

FIG. 1. Bacterial exudate produced on slices of green Kieffer pear fruits 7 days after inoculation with cultures of the organism handled in several ways. A. Ooze that had been collected 6 days after inoculation of pear fruits, dried on cotton thread, and stored at room temperatures for 4 months. B. Bacteria that were taken from agar, immersed in sterile ooze matrix, dried on cotton thread, and stored for 2½ months. C. Slimy growth of organism that was taken from 5 per cent sucrose nutrient agar, dried on cotton thread and stored for 2 months. D. Same bacteria as in C suspended in sterile distilled water and held at room temperature for 3 months.

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[2] The cans used here were originally containers for agar shreds. One perforation in the cover made with a six-penny nail was the only provision for ventilation.

[3] Approximately one half bushel of a sample collected July 27, 1936, was still in fairly good condition on April 8, 1938.

The mounts of the bacterial exudate were prepared by making a thin film on clean slides and allowing it to dry. Carbol fuchsin and Gray's (8) flagella stain showed satisfactorily the capsule-like sheaths surrounding the bacteria in the ooze.

Centimeter lengths of cotton thread proved to be both convenient and satisfactory as a carrier for the fire-blight organism in laboratory experiments involving longevity, thermal death point, and toxicity of bactericides. The thread (No. 40) was cut to length, thoroughly washed in distilled water, and sterilized in vials before use. Bacteria from the various sources—natural matrix, agar, broth, and after washing in the centrifuge—were readily collected, dried, and stored on the threads.

The specimens of pear-stem tissue used for making permanent mounts were fixed in FAA solution (4), dehydrated in butyl alcohol, sectioned 5 to 8 µ thick, and stained with safranin and fast green.

In the majority of the thermal death-point experiments the test medium, nutrient broth, was first placed in a constant temperature bath until adjusted to a given temperature. Then the test bacteria, carried on centimeter lengths of cotton thread, were introduced and subjected to the desired temperature for the customary 10-minute interval. For purposes of comparison in some trials the bacterial specimens were transfered to the test medium first and the temperature brought up to the desired level gradually, and then held for the 10-minute interval. Still other tests were conducted in a dry-air oven.

Toxicity experiments using copper sulphate, hydrated lime, and Bordeaux mixture were conducted with bacteria both from ooze and from culture media for determining their relative resistance to these chemicals. The method employed was the same as that used by Hildebrand (9).

The bacteria were separated from the exudate matrix by centrifuging 4 hours at 3100 r.p.m., followed by passage through a Berkefeld filter. Because of the high viscosity of the ooze when collected from the fruits, it was diluted to one-fourth the original concentration before attempting separation, which made available about 200 cc. of the diluted sterile bacteria-free matrix for each of the three experiments. In two additional trials, where only 10 cc. of ooze was employed, separation and removal of the bacteria were greatly facilitated by using physiological salt solution (0.85 per cent NaCl).

The procedures for the various chemical tests were taken largely from Morrow (14) and the Committee on Editing Methods of Analysis of the A.O.A.C. (5).

EXPERIMENTAL RESULTS

Longevity of Fire-blight Bacteria in Dry Natural Matrix

Virulent cultures of the fire-blight organism were recoverd from the various samples of dried natural ooze for periods ranging from 15 to 25 months after collection in the orchard. In strong contrast to this, bacteria taken from nutrient agar slants or nutrient broth and dried on glass slides or cotton thread usually died within 2 weeks. In one exceptional case the slimy growth of bacteria from 5 per cent sucrose nutrient agar, when dried on cotton thread, survived for slightly longer than 2 months, which suggested that this slime has protective properties worthy of further investigation. Bacterial contaminants, most often yellow, were frequently found in the natural ooze. These had a tendency to obscure the fire-blight organism when grown in liquid culture media. It was found that these contaminating organisms were unimportant if the dried ooze was inoculated immediately into green pear fruits and shoots or plated on agar. While Pierstorff (17), Ark (1), and others have succeeded in isolating pathogenic bacteria from the dried ooze from cankers kept in the laboratory for 2 years or more, it is presumed that the bacteria in all instances were benefiting from the protective action of their natural matrix as in the longevity studies herein reported. This suggests that the bacterial mass produced in the tissues of the affected plant may possess special properties not found in that grown in certain culture media.

Turbidity of Ooze in Relation to Bacterial Longevity

The first ooze appearing on inoculated pear fruits was much more turbid than that appearing later. This was especially noticeable when it was removed at daily intervals. A similar phenomenon has been observed on inoculated blossoms and shoots of pear and apple. There was also a marked decrease in turbidity from day to day when the ooze was allowed to accumulate for about a week under the humid conditions prevailing in moist chambers. This change in turbidity appears to be correlated with the fact that in ooze collected about 14 days after inoculation the bacteria are nearly all dead. As yet the most satisfactory explanation for this rapid death of the organism in the moist ooze is the toxic substance found in the ooze matrix, and discussed later.

Based on these observations it appears that prompt and rapid drying are necessary for the survival of the bacteria in their natural matrix.

Osmotic Pressure Phenomenon and Wilting of Pear Shoots

An experiment was conducted to check on the factor of osmotic pressure in relation to wilting of pear shoots. The test series consisted of a water check, 10 sugar (C.P. sucrose) dilutions ranging by tenths from 0.1 to 1.0 weight molar concentration, and diluted fire-blight ooze. The experiment was conducted in a room at a temperature of about 25° C. According to Shaw (21) the osmotic pressures for this series of sugar concentrations when held at 25° C. are 2.63, 5.15, 7.73, 10.30, 12.94, 15.62, 18.43, 21.25 24.13, and 27.05 atmospheres, respectively. The osmotic pressure of the exudate was determined with the Beckman thermometer and found to be 7.52 atmospheres or 1.61 atmospheres for the dilution (21.4 per cent of the secretion concentration) used in this experiment. Four succulent Kieffer pear shoots were placed in beakers containing 100 cc. of each solution. When observations were made 15 hours later, wilting was most pronounced for the shoots in the ooze where the osmotic concentration was but 1.61 atmospheres, whereas wilting in the sugar series occurred only in weight-molar concentrations of 15.62 atmospheres and above. The same solutions that produced wilting also caused cell plasmolysis when sections of green pear-fruit tissue and strips of the lower epidermis of *Tradescantia* sp. were immersed in the respective solutions. It is evident, therefore, that instead of osmotic pressure some other factor, and presumably a toxic substance, is operating to produce cell plasmolysis and wilting of shoots when immersed in ooze.

The fire-blight organism was observed by Parker (16) to survive but a very short time under conditions of high humidity. Parker's experiments, however, did not exclude contaminating organisms.

Color of Ooze in Relation to Age

The color of the ooze collected from pear fruits at 2-day intervals from the 6th to the 12th day after inoculation was of a relatively uniform cinnamon rufous brown (18). In the absence of contaminants the color of the exudate remained essentially constant, even though the affected pear tissue had in the meantime changed from normal color to deep brown or black. Storage at 3° C. for 4 months, likewise, did not affect the color of the exudate, although the bacteria had died in the meantime. The ooze would, therefore, appear to be a rather uniform substance based on the color criterion. Reports in the literature and elsewhere on color variations in ooze (red, green, etc.) probably are to be interpreted as largely due to contaminating organisms.

Taste of Ooze in Relation to Its Composition

Fire-blight ooze, judged by taste alone, appeared to be relatively free from sugars; but there was evident a faint suggestion of sweetness mingled with a flat, starchy, and slightly acid taste, not easy of definition. In strong contrast the juices extracted from green shoots and fruits, although slightly acid, were definitely sweet, indicating that sugar, supposedly including sucrose, was present. In order to check on the accuracy of taste for the detection of sugars, chemical analyses were made of the diluted ooze matrix and of the juices from green-pear fruits that had been stored for respectively 6 and 18 months at 32° F. The ooze matrix was found to contain approximately 0.5 per cent of sugar (identified as dextrose), which comprised 31 per cent of the dry substance in the matrix. The fruit juices contained 9.60 and 9.28 per cent of sugar and 0.49 and 0.32 per cent of acid, respectively. Reducing sugars were most abundant in the fruit juices. However, approximately one-third of the sugar was sucrose and the bulk of the acid was malic.

Effect on Bacterial Longevity of Prompt and Rapid Drying of Ooze

The effect of prompt and rapid drying on longevity was tested experimentally. Ooze was obtained in the usual manner by inoculating slices of green pear fruits. Six days after inoculation turbid beads of moist exudate up to about 2 millimeters in diameter were accumulating on the cut surfaces of the fruit slices. On the 7th day some of this exudate was transferred to centimeter lengths of sterile cotton thread, dried in a desiccator, and stored in cotton-plugged vials at room temperatures. In the same manner ooze also was collected at intervals of 8, 9, 10, 11, 12, and 14 days after inoculation. For checking purposes the broth culture used for making the inoculations was also dried on cotton threads on the 7th day. The first test for survival was made on the 12th day after inoculation, and viable bacteria were obtained from all the samples, although growth was much slower for the longer intervals. Four days later, on the 16th day after inoculation, viable bacteria were obtained only from the collections made on the 7th day. Two weeks later, or 30 days after inoculation, viable pathogenic bacteria were obtained from the 7-day collection only. The check broth culture survived for 10 days. This isolation experiment was continued and virulent bacteria were obtained from the 7-day collection for approximately 5 months after inoculation.

Another experiment, started about 6 weeks later, gave similar results. The only bacteria surviving for longer than 13 days were from the 7-day collection. It therefore appears that bacteria in ooze show the greatest longevity when collected and dried soon after extrusion. The reason for failure to obtain greater longevity than 5 months by this method may be ascribed in part at least to the relatively thin films of dried ooze that were obtained by the dipping method of impregnation.

Effect on Bacterial Longevity of Moist and Dry Exudate Matrix

Similar effects on longevity to those mentioned above were noted when the blight pathogen that had been produced on agar was subjected to the action of both moist and dry sterile exudate matrix. A 5 mm. loopful of a 3-day-old growth of the bacteria was transferred to about 5 cc. of dilute sterile ooze and held at room temperature. Another loopful was transferred to the same quantity of sterile distilled water. After mixing thoroughly, 2 cu. cm. of each suspension was poured over sterile threads of cotton in vials and dried in a desiccator, the drying operation requiring less than a day. Viability tests were made at short intervals of several days to a week. The experiment was concluded at the end of 3 months. The bacteria from the water suspension that had been dried on cotton thread survived for 16 days, while those left in the water were still alive at the end of 3 months. The bacteria protected by the dry matrix were also alive at the end of 3 months, but those suspended in the moist matrix survived for only 13 days. For checking purposes, ooze produced on pear fruits and dried on cotton threads likewise yielded virulent cultures of the bacteria at the end of the experimental period. Therefore, it appears that the sterile matrix imparts to the bacteria grown on agar the same properties as are possessed by the organism occurring naturally. This experiment was of further significance in demonstrating the lethal effects of the sterile moist ooze matrix on artificial cultures of the bacteria. The period of survival of 13 days corresponded rather closely to that of about 14 days for the natural cultures, as already mentioned.

Reaction of Ooze to Heat

The thermal death point of ooze bacteria produced by inoculating pear fruits was found to be lower than that of bacteria grown on agar or in broth. This indicated that the matrix, rather than giving protection, if anything, made the bacteria more sensitive to heat. In one experiment the ooze bacteria collected 7 days after inoculation were viable following a 10-minute exposure at 40° but failed to survive at 43° C., whereas bacteria of the same age from the same cultures grown on nutrient media survived at 46° but not at 48° C. In another and similar experiment the bacteria in ooze 8 days after inoculation withstood 40° but not 42° C., whereas those from the same culture grown in nutrient broth for 12 days survived a 10-minute exposure at 46.0° but not at 47.5° C. In a third experiment ooze bacteria failed to survive at 43°, while those from nutrient broth withstood 47° but not 48° C. Age also seemed to influence the resistance to heat of this organism grown in culture media, since 1-day-old bacteria withstood 47.5° C., whereas 3-day-old bacteria failed to survive this temperature. Other trials were conducted in the dry-air oven, but neither the ooze bacteria nor those grown on nutrient media and dried on cotton thread survived a 10-minute exposure at 50° C., the lowest temperature used. Therefore, it appears that the protecting influence of the natural matrix was not operative in heat resistance and, if anything, it seemed to increase the thermolability of the bacteria.

Reaction of Ooze to Bactericides

The fire-blight organism, when in its natural matrix, was found to be more sensitive to bactericidal action than when grown on culture media The bactericidal materials employed in reaching this conclusion were copper

TABLE 1.—*Summary of laboratory studies to determine the relative resistance to bactericidal action of the fire blight organism when grown (a) on pear fruits and (b) on culture media*

Spray materials tested	Formula	Parts by weight	Source of bacteria	Number of trials	Range of concentrations[a] killing bacteria within:	
					10 min.	30 min.
Copper sulphate	1–25	1–200	Ooze	4	1/1	1/16
			Agar culture	4	1/.5	1/1
Lime (hydrated)	3–25	3–200	Ooze	4	1/32	1/64
			Agar culture	4	1/2	1/16
Bordeaux mixture	1–3–50	1–3–400	Ooze	4	1/16	1/32 to 1/128
			Agar culture	4	1/4 to 1/8	1/8 to 1/32

[a] The formulae are considered as unity, or 1/1.

sulphate and hydrated lime alone and when combined to form Bordeaux mixture. From the results, summarized in table 1, it is evident that, although handled under identical conditions, the bacteria in the ooze were always killed at more dilute concentrations of the bactericides than were those of the agar cultures.

The reason for the greater sensitivity of bacteria in the ooze to bactericides, as well as to heat, as already discussed, has not been fully established. There is, however, evidence that some substance is present in the ooze matrix not found in significant amounts in artificial culture media, which increased the sensitivity of the organism to its surrounding environment. Moreover, this suspected substance may possibly be the mechanism by which the organism obtains food from the protoplast of the suscept cell.

Ooze as a Carbon Source in Culture Media

The pathogen is able to utilize the diluted sterile exudate as a carbon source when grown in synthetic carbohydrate medium (Society of American Bacteriologists—1923–1931) (22). One cc. of the ooze matrix was added to 10 cc. to this medium. It seems no serious toxic effects were involved at this concentration, since the bacteria were still alive at the end of a month. Also, chemical tests made at this time showed no trace of sugar.

Reaction of Ooze to Dyes

Fire-blight bacteria stain unevenly with carbol fuchsin dye when embedded in their natural matrix. The bacteria were prepared by simply making a thin smear with the ooze on a slide and staining after air drying. This phenomenon is illustrated by stained preparations made from ooze collected 6 and 12 days after fruit inoculation (Fig. 2, A and B). The dye was taken up most readily at the poles of the cells, and this tendency was most marked for the older sample (Fig. 2, B). Rosen (19) observed a similar phenomenon with the crown-gall organism, and certain species of bacteria other than the plant pathogens are known to behave in a similar fashion. The method of handling the bacteria was the same as that used by Rosen, no heat being applied in fixing them to the slide. What significance, if any, is to be attached to the above observation would probably depend on the writer's concept of the nuclear question in bacteria. Knaysi (1938), in his paper on the cytology of bacteria, discusses this point.

The same stained preparations showed the bacteria to be surrounded by a capsule-like sheath (Fig. 2). A similar, if not identical, phenomenon is figured in Nixon's (15) and Rosen's (20) illustrations. What Nixon terms "cysts" were probably dried spherical masses of the bacterial exudate, which, because of the irregular staining of the bacteria, may have given the impression of granules instead of cells.

There seems to be some question as to the identity of the sheath seen about the cells, since this coating, which is apparently nothing other than matrix, was removed by centrifuging at high speeds in water or physiolog-

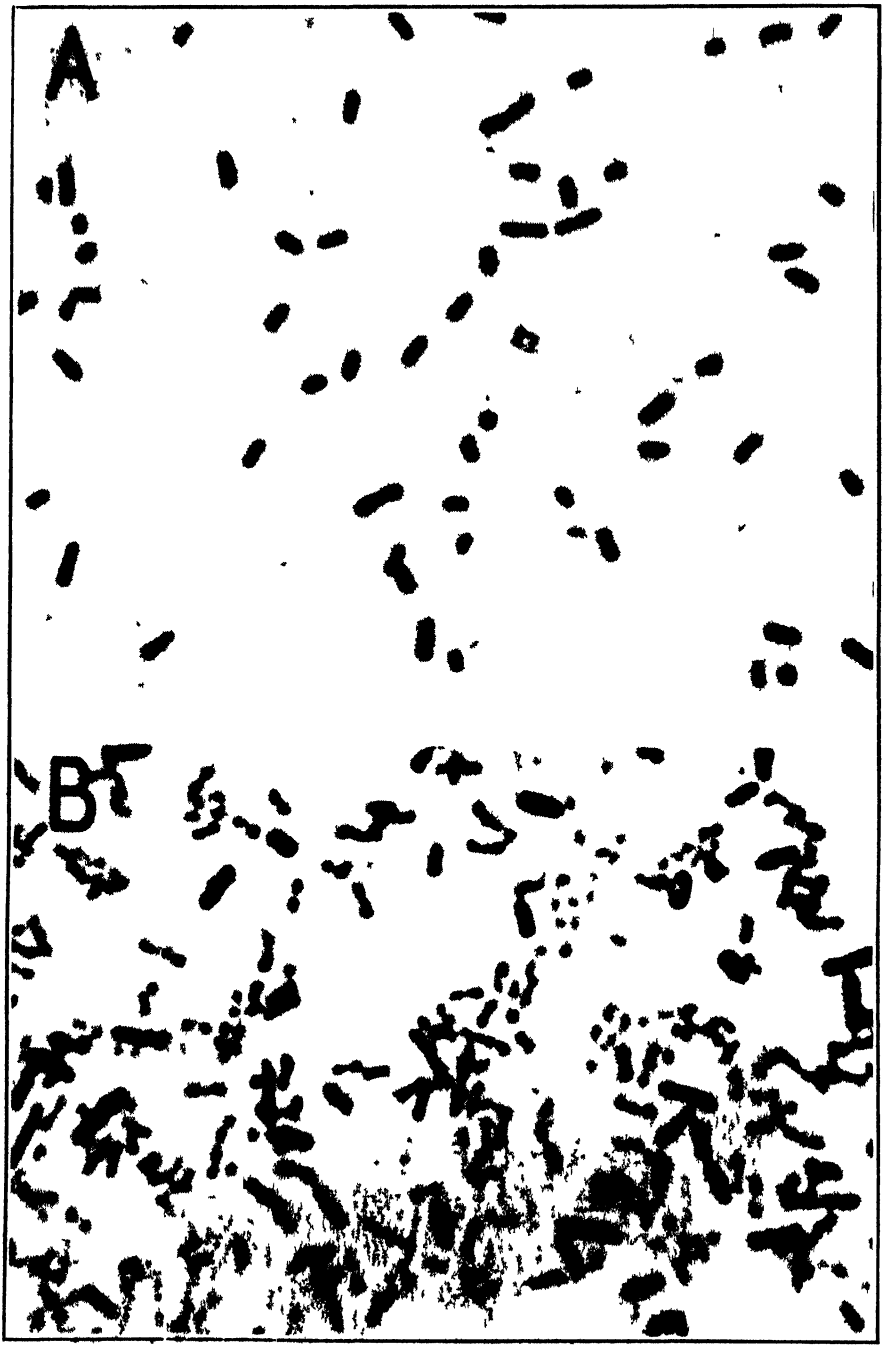

FIG. 2. Preparations of fire blight ooze taken from blighting green pear fruits in the laboratory and stained with carbol fuchsin. The bacteria stained unevenly, a capsule-like sheath surrounds the individual cells. A. Ooze collected 6 days after inoculation. × 3000. B. Ooze collected 13 days after inoculation. × 2880.

ical salt solution. Whether or not this removable layer should be considered a form of capsule (7), it is significant that the dried films of the natural matrix imparted specific protective properties to the organism that were not observed when it was grown in culture media. There was, however, as already mentioned, the exceptional instance in which the dried slimy bacterial growth from 5 per cent nutrient sucrose agar survived for about 2 months, but further work is needed to clarify this point. While descriptions in Bergey's Manual (2) and in Elliott's Manual (6, pp. 18 and 19) and elsewhere have not included capsule formation as a characteristic of the fire-blight organism, it should be pointed out that these descriptions were all based on bacteria grown on culture media. It may be worthy of note here that what appear to be capsules have been observed by the writer in the fire-blight pathogen for approximately 2 per cent of a total of over 100 artificial cultures stained with Gray's flagella stain.

Ooze Contains a Toxic Substance

When the cut ends of succulent pear shoots were immersed in ooze or sterile ooze matrix, the shoots wilted, indicating the presence of a toxin. Heretofore, only Pierstorff (17) had been successful in demonstrating toxic action to suscept tissue with extracts from infected pear fruits.

The first experiment was conducted by placing 2 shoots in each of 3 vials containing, respectively, water alone, sterile ooze matrix of ¼ the secretion concentration, and sterile ooze matrix diluted to 1/50 the secretion concentration. In succeeding experiments only the more concentrated matrix solution and the water check were employed. The stems were thor-

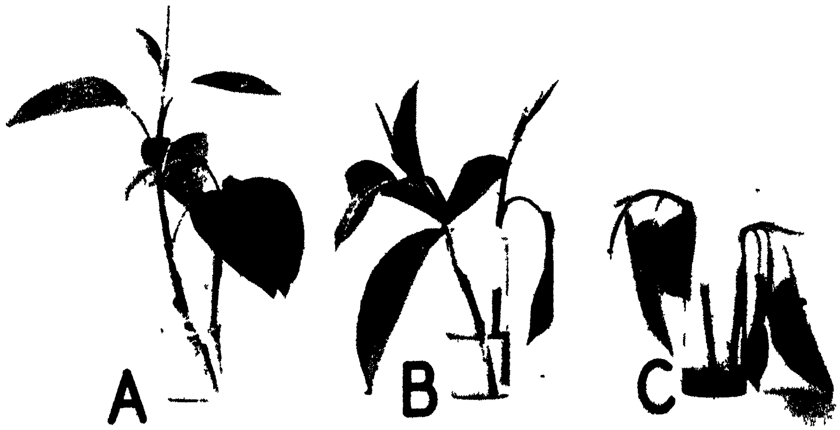

FIG. 3. Toxicity of the sterile ooze matrix of fire blight to pear shoots. Photographed at the end of 2 hours. A. Water check, no wilting. B. Slight wilting, ooze matrix diluted to 1/50 of the secretion concentration. C. Pronounced wilting, ooze matrix diluted to 1/4 the secretion concentration.

oughly washed in tap water and cut under water before placing in the vials. The progress of wilting at the end of 2 hours is illustrated in figure 3. It will be noted that the shoots immersed in the more concentrated dilution of the ooze matrix were severely wilted, which is in striking contrast to the slight wilting found in the more dilute matrix and to no wilting at all in the water checks. This experiment was repeated daily for a week with similar results.

The wilting was accompanied by necrosis at the cut ends when succulent shoots were permitted to remain in the matrix for several hours. When left in the matrix for 18 hours, the length of the blackened ends varied from 2 to 10 mm. and the necrosis was most pronounced for the younger, more succulent shoots. After removing about one-half inch of the lower portion of the stem by cutting under water, the top portion was placed in distilled water to test for recovery from wilting. Of a total of 12 shoots thus treated all but 3 effected at least a partial recovery in 2 days. Those failing to respond at all were of the younger, more succulent kind.

Permanent mounts prepared from the lower portions of the stems of wilted shoots showed plasmolysis of many of the suscept cells. The specimens used consisted of stem lengths up to one-half inch, which included the discolored portion at the base of the stem plus an equal amount of the nondiscolored portion above Although slightly less pronounced at the end most remote from contact with the toxic solution, cell plasmolysis extended for the entire length of all of the sections examined. Besides plasmolysis, deeply staining material had occluded over the cut surface immersed in the toxic solution, being thickest over the vessels (Fig. 4). This accumulation has not been identified, but is thought to be the remnants of the content of cells killed or seriously injured by the toxin.

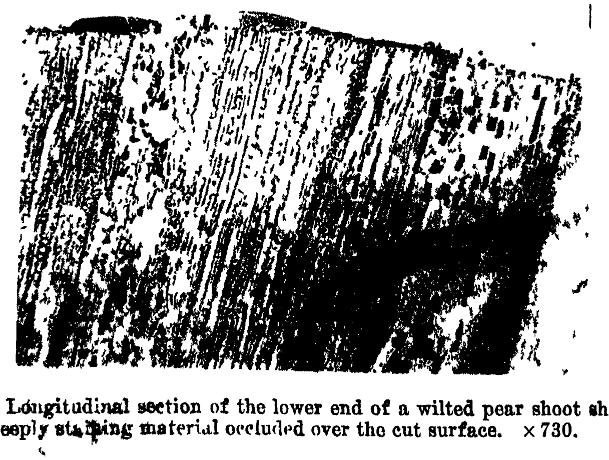

FIG. 4. Longitudinal section of the lower end of a wilted pear shoot showing besides plasmolysis deeply staining material occluded over the cut surface. × 730.

Plasmolysis was observed also under the microscope when free-hand sections of green pear fruit tissue were immersed for several hours in sterile ooze matrix. When transferred back to water many of these cells failed to recover. It was concluded, therefore, that false or toxic plasmolysis had occurred. The death or permanent injury produced is attributed to a toxic substance resident in the ooze matrix. This experiment was repeated with similar results.

Plasmolysis was similarly observed followed by loss of color from the cells when strips from the lower epidermis of *Tradescantia* sp. were immersed in the ooze matrix. Here plasmolysis was more rapid, becoming apparent within a short time and at 1 hour was well advanced. In about 2 hours it had apparently reached its maximum. If allowed to remain in the matrix solution for one to several hours longer the color disappeared from within the plasmolyzed cells. Essentially the same results were obtained by repeated experiment.

The toxic principle in the ooze matrix was not destroyed by heating in the Arnold steamer for several hours or by heating in dry-air oven at 100° C. for 3 months. This seems to be good evidence that the toxic principle is thermostable.

Numerous experiments on the chemistry of the ooze matrix have thus far been unsuccessful in determining the identity of the toxic principle.

DISCUSSION AND CONCLUSIONS

The fact brought out in this investigation that the exudate must be promptly and rapidly dried for long survival of the bacteria is in accord with the observations of Blake (3) that fire blight is usually more severe during hot, dry seasons than during cool, wet seasons, under New Jersey conditions. While in New York such dried exudate has been observed to persist in the orchard for intervals of several weeks under dry weather conditions, and in the laboratory for even 25 months, no source of primary inoculum other than cankers has yet been found. Survival of the bacteria in soil for relatively long intervals in a dry climate (1, 23) attests the protective qualities of the dry ooze matrix. On the other hand, the rapid death of the bacteria in uncontaminated ooze under humid conditions in the laboratory, indicates that they do not long survive therein under field conditions.

This study seems to indicate that the so-called "zoogloea" of Nixon (15) probably are nothing other than fire-blight ooze *in situ* in diseased tissue. Moreover, the capsule-like coat surrounding the bacteria is undoubtedly ooze. According to Miller (13), bacteriologists usually apply the term zoogloea only when the slimy matrix embedding the bacteria has been secreted by the bacteria themselves. Since the slimy matrix is associated with growth in suscept tissue and not in culture media, the use of the term thus defined would seem to be unwarranted. Moreover, the fact that 31 per cent of the matrix is sugar seems to be a further argument against its lone bacterial origin.

The observation that the ooze matrix, when in the moist state, is a very poor environment for the survival of these bacteria suggests the presence of a substance deleterious to the pathogen. Bacteria grown on culture media and introduced into the sterile ooze matrix were likewise short lived. Other observations on the greater sensitivity to heat and to bactericidal action of the ooze bacteria in contrast to those grown on culture media furnished further evidence of the presence of a deleterious substance. The evidence accumulated strongly indicates that infection by the fire-blight organism is effected by a killing of the suscept cells by a toxin secreted by the organism growing in the intercellular spaces.

The presence of a toxic substance in the ooze seems to offer the only explanation of the wilting of cut pear shoots when placed in solutions of bacteria-free exudate.

Rosen's (20) recent publication on the "Life span and morphology of fire-blight bacteria, as influenced by relative humidity, temperature, and nutrition" sheds considerable light on the topic under discussion. The reader is referred to his most interesting report for further comment.

SUMMARY

This study deals with the nature of the exudate or ooze occurring in fire blight.

Virulent cultures of the pathogen were recovered from different samples of dried natural ooze during periods ranging from 15 to 25 months after collection in the field, whereas the organism grown in culture media and dried ordinarily never survived for so long as one month.

The ooze used in the majority of these studies was produced by inoculating green pear fruits under aseptic conditions. A yield of about 1 cc. of moist ooze per fruit was obtained.

The first ooze appearing on inoculated pear fruits is much more turbid than that appearing later, and the tendency for moist ooze to clear with age is correlated with rapid death of the bacteria. Prompt and rapid drying were found necessary if the bacteria are to survive for longer than 2 weeks in their natural matrix.

The color of the ooze collected from pear fruits is of a uniform cinnamon rufous brown.

Judging from taste alone, fire-blight ooze appears to be relatively free from sugars when compared with the slightly sweet juices of green pear fruits, but chemical analyses show that a sugar identified as dextrose comprises 31 per cent of the dry substance in the matrix.

The effect of prompt and rapid drying on the longevity of the pathogen was demonstrated experimentally when only the ooze collected at the shortest interval of 7 days after inoculation survived for longer than 2 weeks (approximately [illegible] months).

Similar effects on longevity were noted when the blight pathogen, which had been grown on agar, was subjected to the action of the sterile exudate matrix.

The thermal death point of ooze bacteria from pear fruits was found to be lower than that of bacteria grown on agar or in broth.

The fire-blight organism, when in its natural matrix, was found to be more sensitive to bactericides than when grown on culture media.

The fire-blight organism is able to utilize the diluted sterile exudate as a carbon source when grown in synthetic medium.

Fire-blight bacteria stain unevenly with carbol fuchsin dye when embedded in their natural matrix. The same stained preparations show the bacteria to be surrounded by a capsule-like sheath, which could be removed by centrifuging.

Diluted fire-blight ooze having an osmotic pressure of 1.61 atmospheres produced wilting of pear shoots and plasmolysis of the cells, whereas in pure sucrose solutions osmotic pressures of 15.62 atmospheres and above were required to produce these effects. This indicates that some factor other than osmotic pressure was operative in the ooze.

When the cut ends of succulent pear shoots were immersed in sterile ooze matrix, the shoots wilted indicating the presence of a toxin. The wilting was accompanied by necrosis at the cut ends. Permanent mounts show plasmolysis of the suscept cells. Plasmolysis was observed also under the microscope when free-hand sections from green pears were immersed for several hours in the sterile matrix. Plasmolysis was similarly observed followed by loss of color from the cells of the lower epidermis of *Tradescantia* sp.

The toxic substance is thermostable, but numerous experiments have thus far been unsuccessful in determining its identity.

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TOMATO TIP-BLIGHT VIRUS[1]

J. A. MILBRATH

(Accepted for publication Sept. 10, 1938)

An important disease of tomato, known as tip blight (5), has been present in Southern Oregon for several years. A full account of the history, description, and certain economic features have been given (6). The present paper describes the causal agent as a distinct virus and compares it with other viruses affecting Solanaceous plants.

SYMPTOMATOLOGY

Since a full description of symptom development of tip blight has already been given (6), only a brief discussion of the outstanding symptoms will be presented here. The most prominent symptom of tip blight is the pronounced blighting and blackening of the terminal shoots of affected plants (Fig. 1). The dead tips stand upright above the living foliage in a very characteristic manner. The leaves of affected shoots become spotted with either a few large black necrotic lesions or else many small ones (Fig. 2, A). These lesions are rounded, somewhat zonate and conspicuously black. They increase in size and tend to coalesce, causing a collapsing and wilting of the entire leaf. The margin between living and dead cells is sharp, and the necrosis extends from one surface of the leaf to the other. The small green fruits become rough and pitted with internal necrotic pockets; eventually, they fall from the plant without ripening. Larger fruits ripen unevenly and become discolored with various patterns of yelllow and orange. Large green fruits often exhibit sub-epidermal necrosis, which externally appears as concentric brown bands.

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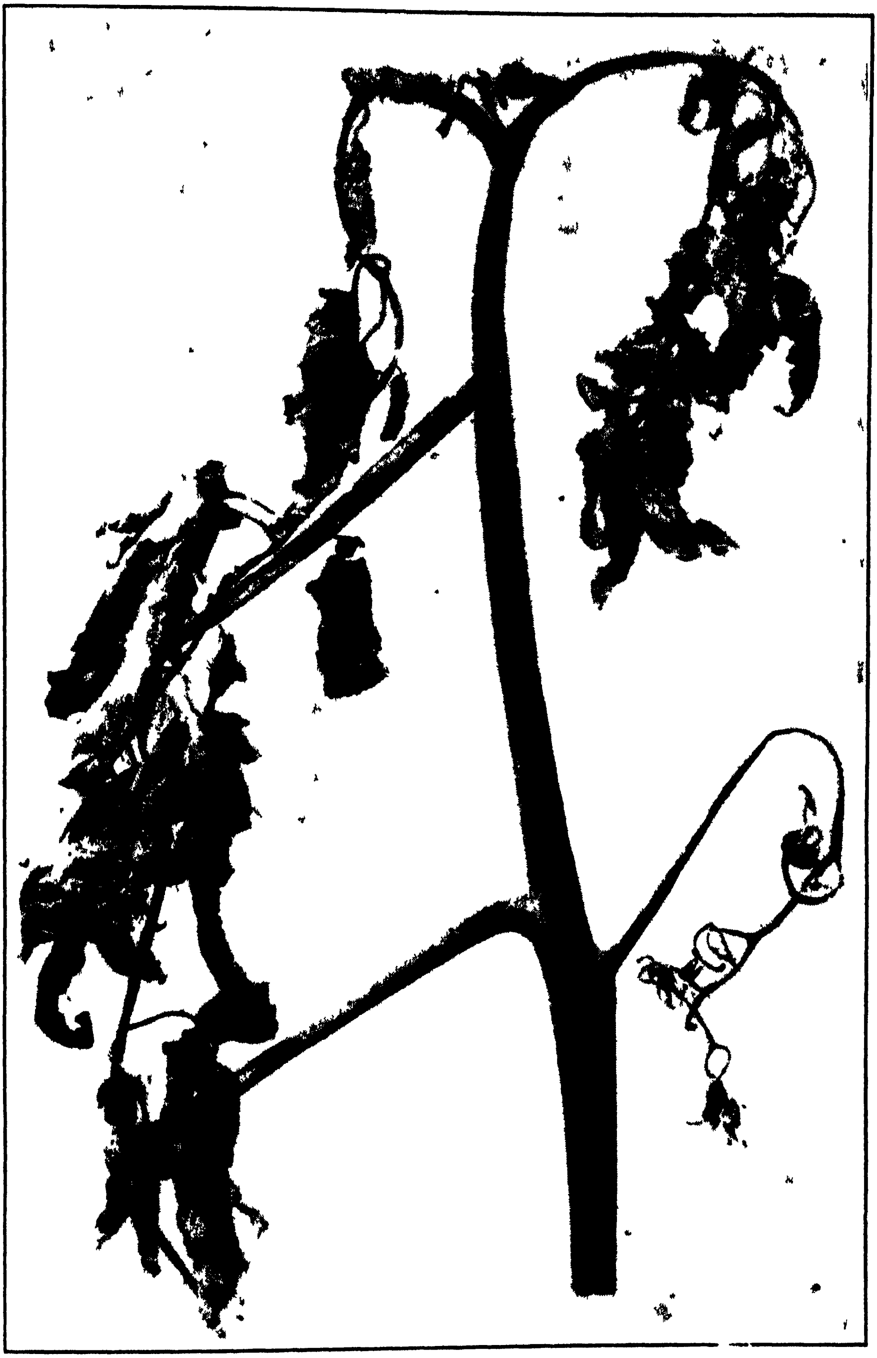

FIG. 1. The terminal portion of a branch of a tomato plant affected with the tip-blight virus. The withered leaves, the stem streaking and the blighted tip are characteristic.

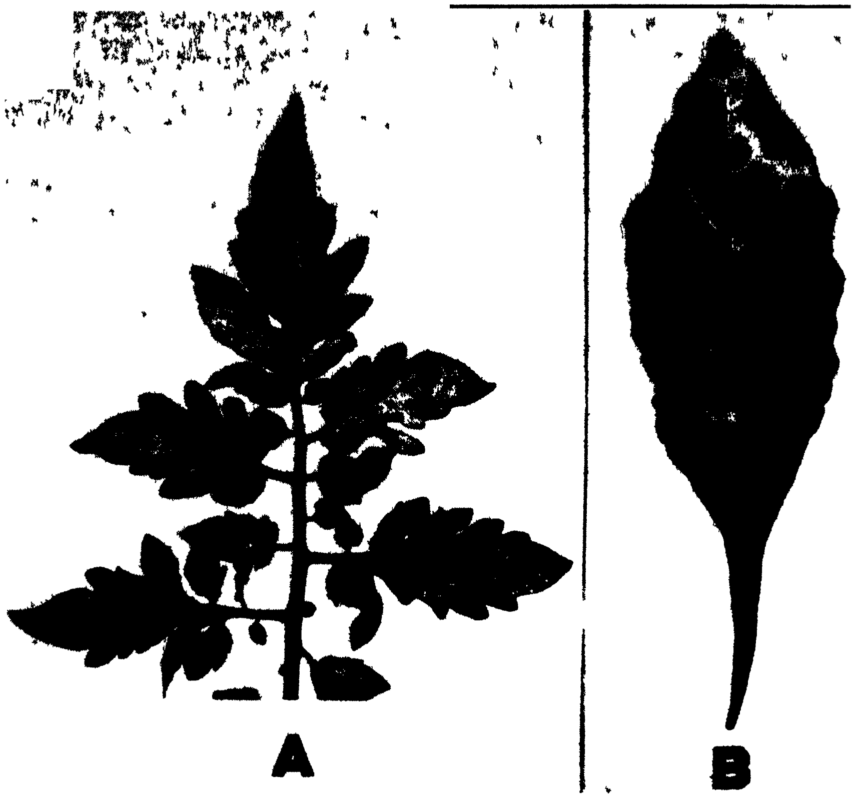

FIG 2 A Typical symptoms of the tip blight virus on a leaf of Indiana Baltimore tomato B Black, local lesions produced by the tip blight virus on *Solanum capsicastrum*.

ETIOLOGY

It was suggested by McWhorter and Milbrath (5) that tip blight probably was caused by 2 viruses Spotted wilt and an undetermined virus Further studies have shown that tip blight is caused by a distinct virus, and that spotted wilt is present only occasionally. It is suggested that the name tip blight be retained as the common name of this disease and that the virus be known as tip-blight virus until a uniform method of naming plant viruses has been adopted The nature and properties of the virus are discussed below.

Transmissibility

Grafting—The virus is readily transmitted by grafting The most efficient method is a straight side graft, held in place by thin rubber bands, and covered with tinfoil to prevent desiccation Tissue insertion and inarch grafting also will transmit the virus Necrosis has developed on the tips of healthy tomato plants in 7 days after the infected scion was placed in graft position. Since it takes a similar period for the secondary symptoms to appear after the formation of local lesions by mechanical inoculation, the virus must move from the infected scion into the healthy stock before organic union has taken place.

Mechanical.—Mechanical transmission of the tip-blight virus is difficult. Light or vigorous rubbing of the leaf surface with cotton swabs (4) or glass spatulas (9), dipped in juice extracted from diseased plants, have failed to transmit the virus. Scratching the leaves with needles through drops of viruliferous juice, or pricking the leaves through diseased leaves with Sein's multiple needles (10) have not transferred the virus. The soaking of cotton lint in the virus, and inserting this inoculum in the leaves or leaf axils, a method successfully used for transferring spotted wilt by Doolittle and Sumner (2), has not been successful with the tip-blight virus. The only mechanical method of transmission that has given a fairly high percentage of virus transfer has been the carborundum method, as reported by Rawlins and Tompkins (7). At temperatures of 70° F. or lower, nearly 100 per cent transfer of the virus can be obtained by this method, provided young, vigorous plants are inoculated with juice taken from plants recently affected with the tip-blight virus. Inoculations must be made within a few minutes after the juice is extracted.

Hypodermic Needle.—A low percentage of transfer of the tip-blight virus was obtained, when the infectious juice was injected into the stems of young vigorous tomato plants. Five plants became infected with tip blight when 27 plants were inoculated in this manner.

Insect Transmission.—Preliminary experiments on insect transmission have shown that thrips are the likely natural disseminating agents. These tests (6) point to *Thrips tabaci* as being the chief infective species, but other species of thrips may be vectors. Black, necrotic, local lesions develop at the points of thrips feeding 6 to 7 days before typical symptoms develop on the terminal leaves.

Soil Transmission.—Direct experiments and field observations have failed to indicate any soil transmission of the virus.

TABLE 1.—*Transmissibility of the tip-blight virus*

Plant inoculated	Method of inoculation	No. of plants inoculated	No. of plants positive	Percentage of transfer
Tomato	Side graft	31	25	80.64
Tomato	Sein's needle	20	0	0
Petunia	Sein's needle	12	0	0
Tomato	Cotton swab	71	0	0
Petunia	Cotton swab	11	0	0
Datura	Cotton swab	17	0	0
Tobacco	Cotton swab	12	0	0
Tomato	Glass spatula	20	0	0
Tomato	Cotton insert	21	0	0
Tomato	Carborundum	307	162	52.76
Petunia	Carborundum	36	13	36.11
Datura	Carborundum	35	20	57.14
Tobacco	Carborundum	34	28	82.35
Tomato	Hypodermic needle	27	5	18.51
Tobacco	Hypodermic needle	5	0	0

Seed Transmission.—Hundreds of plants have been grown from seed derived from infected tomato fruits. None of these plants developed tip blight.

Physical Properties of the Virus

Longevity in Vitro.—Tip-blight virus is inactivated in extracted juice in a very short time. Undiluted juice loses its power of infection in less than an hour at 65° F. or higher. Juice exposed to the air in flat open dishes, or juice that it agitated loses its infectiousness more rapidly.

TABLE 2.—*Longevity of tip-blight virus in vitro*

Duration of aging, in minutes	Container	No. of plants inoculated	No. of plants positive
0	Test tube	5	5
15	" "	5	4
30	" "	5	5
45	" "	5	2
60	" "	5	0
30	Open vessels	5	4
45	" "	5	0
60	" "	5	0
75	" "	5	0

Thermal Death Point.—Tip-blight virus has a very low thermal death point. In measuring the thermal death point, 15 to 20 cc. of crude expressed viruliferous plant sap in thin test tubes were used. The tubes were immersed in a water bath for 10 minutes at the desired temperature and then cooled immediately in cold water. Check tubes were held for 10 minutes at greenhouse temperature to compensate for the loss in activity caused by aging. A similar experiment was conducted in which the plant sap was heated in 250 cc. flasks instead of the test tubes.

After heating the juice at 40° C., 7 of the 7 plants inoculated became infected, while another lot of juice, heated to 41.5° C., failed to infect any of

TABLE 3.—*Thermal death point of the tip blight virus*

Temperature of heating	Type of container	Plants inoc.	Plants positive
(°*C.*)		(*No.*)	(*No.*)
32.5	250 cc. flask	5	5
36.0	" "	5	1
Check	" "	5	4
35–36	" "	6	0
40–41	" "	6	0
Check	" "	6	2
36	" "	6	0
40	" "	6	0
Check	" "	6	6
Check	Test tube	7	7
40	" "	7	7
41.5	" "	7	0
42.5	" "	5	0
Check	" "	5	5

the 7 plants inoculated. The thermal death point for the tip-blight virus appears to be between 40° and 41.5° C. Juice heated in 250 cc. flasks becomes inactivated between 35° and 40° C., probably close to 36° C.

Dilution End Point.—The tip-blight virus loses its infectious properties readily on dilution with water, without aging. In testing the dilution end point large succulent leaves of Connecticut Havana tobacco were used as an index. Undiluted juice produced an average of 17.2 local lesions per leaf, juice diluted 1 to 1 produced 12.4 local lesions per leaf, and that diluted 1 to 20 produced only 5.6 local lesions per leaf. Juice diluted 1 to 50 or more failed to produce any local lesions on the inoculated tobacco leaves.

Desiccation.—No inoculations have been made from juice extracted from dried leaves taken from tip-blighted plants. Experiments have shown that mechanical transmission of the virus is possible only when using leaves recently affected by the virus. Apparently, the virus is inactivated as the cells die.

Ultrafiltration.—No attempts have been made to determine the size of the virus particles by ultrafiltration. Such experiments cannot be conducted until some method is devised that will prevent the rapid inactivation of this virus during the time required for a filtration test.

Differential host plants

Many species of plants, involving several families, have been inoculated with the tip-blight virus. These inoculations have shown that on certain plants the virus produces symptoms that differentiate it from all other tomato viruses. With few exceptions, black, necrotic, local lesions were produced on susceptible plants. On many plants this was the only symptom. With the exception of the nasturtium plant, the secondary symptoms were consistently necrotic leaf spotting, stem streaking, and tip blighting, without any mottle or bronzing appearing on the foliage. Table 2 summarizes the outstanding symptoms noted for the susceptible plants included in these studies.

Tomato, *Datura, Solanum capsicastrum,* Bliss triumph potato, nasturtium, and tobacco are the most effective differential host plants. A brief discussion of the outstanding symptoms produced by the tip-blight virus on these plants is given below.

Tomato.—Tip blight virus produces its most characteristic symptoms on the tomato plant. Stem streaking and circular necrotic leaf lesions, (Fig. 2, A) followed by a complete blighting of the terminal tips (Fig. 1) developed on all the varieties of tomatoes inoculated. Black, necrotic, local lesions follow either insect or artificial inoculation. Bronzing and mottling were consistently absent. This combination of symptom manifestation seems unlike that of any other tomato virus that has been described, and readily differentiates the virus of tip blight.

Datura.—Local necrotic lesions may or may not be present on the inoculated leaves of *Datura;* the deciding factor is the age of the leaf at

TABLE 4.—*Symptoms produced by tip-blight virus on various plants*

Test plants	Local lesions	Secondary lesions	Tip blighting	Leaf mottle	Stem streaking	Chlorosis
Lycopersicum esculentum	M[a]	S	S	–	S	–
Datura stramonium	M	S	S	M	–	–
Nicotiana tabacum	S[a]	S″	S″[a]	–	S″	–
Petunia	M	–	–	–	–	–
Tropaeolum	–	M	–	S	–	M
Zinnia	S	M	–	–	M	–
Nicotiana glutinosa	S	–	–	–	–	–
Solanum tuberosum	S	–	–	–	–	S′[a]
Amaranthus retroflexus	M	–	–	–	–	S
Solanum nigrum	M	S	S	–	S	–
Nepeta cataria	M	M	–	–	–	–
Pisum sativum	S	–	–	–	–	–
Lactuca sativa	M	S	–	–	–	–
Lactuca scariola	M	S	–	–	–	S′
Malva rotundifolia	M	–	–	–	–	M′
Solanum capsicastrum	M	–	–	–	–	M′

[a] S, severe; M, mild; ″, when present; ′, on inoculated leaf only.

the time of inoculation. If local lesions are formed, they are few, irregular in outline, and black. Secondary lesions develop on the younger foliage. At first, they are faintly chlorotic, circular areas with a small necrotic center; later, the necrosis spreads until the entire spot becomes a black lesion that coalesces with adjoining lesions (Fig. 3, C). Eventually, the leaf is severed at the abscission layer and the leaf falls from the plant. This continues until all the leaves have fallen and only the green stem remains. Although the stems may remain green for several months, no new growth develops, for the virus has destroyed the terminal meristematic areas.

Solanum capsicastrum.—Large, black, necrotic local lesions bordered with a faint chlorotic margin, are the only symptoms produced when *Solanum capsicastrum* is inoculated with the tip-blight virus (Fig. 2, B). There is no indication of the highly developed ring spot obtained when this plant is inoculated with the spotted-wilt virus (14). Rarely more than 1 or 2 lesions develop per leaf. No secondary symptoms were apparent on any of the plants inoculated.

Bliss Triumph Potato—Five to 6 days after inoculation, black, necrotic lesions begin to form on the inoculated leaves of Bliss Triumph potato. (Fig. 3, B). These continue to enlarge until they are nearly one-half inch in diameter. The progression of the virus is such that the necrotic area assumes a concentric formation. The concentric rings are formed by slightly raised, dark, narrow ridges that occur at regular intervals. The remaining area of the leaf soon becomes chlorotic and the leaf falls from the plant. The virus has not been observed to become systemic.

Nasturtium.—The inoculated leaves of the nasturtium plant show considerable mechanical injury and very indefinite effects of the tip-blight virus. The leaves become yellow or orange, and eventually they wither and die.

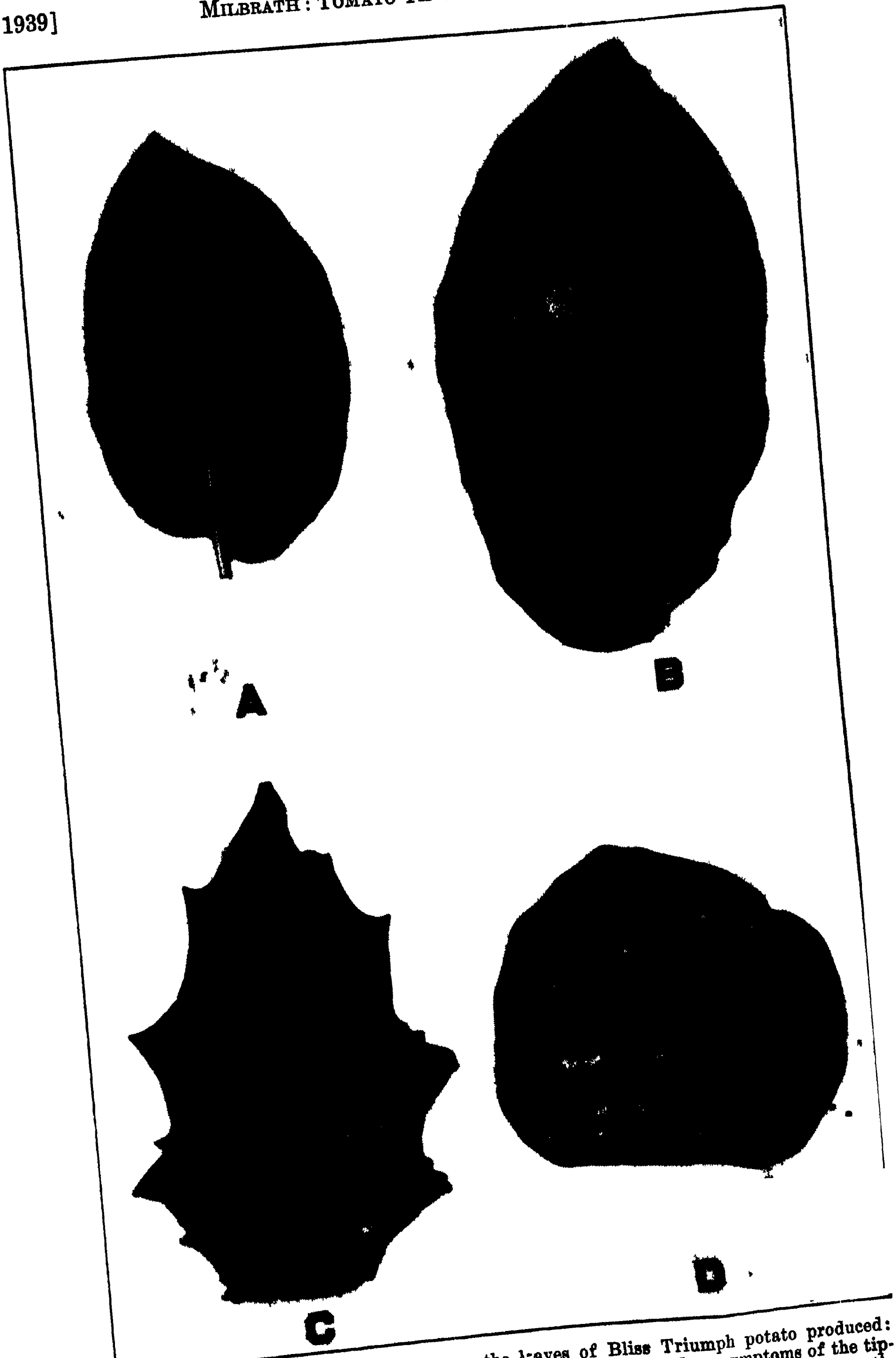

FIG. 3. A and B. Local lesions on the leaves of Bliss Triumph potato produced: A, by spotted wilt virus; B, by tip blight virus. C and D. Secondary symptoms of the tip-blight virus; C, on *Datura*; D, on nasturtium. Note the abundance of black, necrotic lesions on the nasturtium.

The secondary symptoms are a pronounced mottle, which either remains in patches or diffuses as a lighter green color over the entire leaf. On some leaves there is a tendency for the mottle to appear as circular areas with concentric lines about the margins. Other leaves develop numerous, small necrotic areas with pale green margins. (Fig. 3, D). These spots are circular in outline and black in color. The affected leaves soon wither and fall from the plant, but the meristematic regions are not destroyed and new leaves continue to develop. The entire plant is seldom killed.

FIG. 4. Local lesions produced by the tip-blight virus on the leaves of Connecticut Havana tobacco.

Tobacco.—Black necrotic local lesions develop on Connecticut Havana tobacco 4 to 5 days after inoculation with tip-blight virus. (Fig. 4.) Occasionally, secondary necrotic lesions develop on the younger foliage, but, for the most part, the virus does not become systemic. The secondary and primary lesions are similar, but the former are more abundant and much smaller. The necrosis never follows the veins, but the lesions may form on the veins or between them.

DIFFERENTIATION FROM CERTAIN OTHER TOMATO VIRUSES

The majority of viruses affecting tomato plants are so different from the tip-blight virus that a comparison would be superfluous. The viruses responsible for Datura virus 1 (17), ring spot (3), die-back streak (11) and spotted wilt are sufficiently like tip-blight virus in their properties or effects to warrant special discussion.

Datura Virus 1.—The new virus reported by Smith (16) resembles tip-blight virus in that both produce necrotic local lesions, extreme leaf necrosis and stem streaking on the tomato plant. The streaks formed by the Datura virus 1 is at the soil line, which is quite different from that of the tip-blight virus. The type of local lesions developed by the 2 viruses on *Nicotiana glutinosa* and the type symptoms on *Datura* are distinct. Datura virus 1 has a thermal death point of 80° C., dilution end point of not more than 1–10,000 and the resistance to aging is measured in weeks. These physical properties differ greatly from those of the tip-blight virus.

Ring-Spot Type of Virus.—Imle and Samson's (3) abstract briefly descirbes symptoms somewhat similar to those of tip-blight virus on tomato. The physical properties, however, clearly demonstrate that the 2 viruses are different. The thermal death point of the ring-spot virus is 56–58° C., dilution end point 1–500, and the resistance to aging is 21 to 27 hours.

Die-back Streak.—Shapovalov (11) has reported a disease of tomatoes in California that he named die-back streak. It has been called die-back streak in several papers, (11), (12), (18), but later, Shapovalov (13), in speaking of die-back streak of the Pacific Coast, says it is "apparently identical with English and Australian spotted wilt." It is apparent from his brief descriptions that he is dealing with some combination virus complex of which spotted wilt is one of the factors; his descriptions do not coincide with those of typical spotted wilt. Kenneth M. Smith (15) has shown that spotted wilt, in combination with mosaic, will produce streak symptoms. However, from Shapovalov's descriptions of die-back streak, mosaic virus seems to be wanting, as he did not obtain local lesions when inoculating *Datura*. Instead, he reports a blotchy, puckered, coarse mosaic of the leaves with no necrosis; these are the symptoms produced by spotted wilt. The symptoms of tip blight on *Datura* have already been described. Shapovalov's descriptions of the die-back streak on tomato includes the symptoms of tip blight, but, in addition, he reports bronzing as a frequent symptom. It is quite possible that Shapovalov's die-back streak is induced by a mixture of the spotted-wilt and tip-blight viruses.

Spotted Wilt.—Parallel inoculations with tip-blight and spotted-wilt viruses on the same variety of tomato, under the same conditions, have consistently induced dissimilar symptoms that clearly distinguish the two. However, the similarity of symptoms on tobacco, nasturtium, petunia and on tomato fruits, the ability to affect a similar host range, the transmission by the same insect vector, and a close agreement in physical properties, suggest a close relationship of the 2 viruses. In these studies there has been no evidence to support the theory that one of the viruses might be only a component part of the other. There has been no tendency for either virus to change from one form to the other. The outstanding differences between the viruses may be summarized as follows: On tomato the spotted wilt virus is characterized by a pronounced bronzing of the leaves, an extreme dwarfing of the growing point, and the development of a mottled new growth. The plants are seldom if ever killed. The tip-blight virus is characterized by the black necrotic leaf spotting on tomato. Neither bronzing nor mottling has ever been noted on any of the varieties of tomatoes inoculated with the tip-blight virus. No new growth develops, as the entire tip is killed within a few days after the first symptoms appear. Cross sections show that the bronzing of tomato leaves by spotted-wilt virus is the result of a necrosis of the epidermal cells. Leaves affected by the tip-blight virus fail to show this localization of the necrosis; the cell breakdown occurs from one epidermis to the other at equal rate (6). The spotted-wilt virus causes a ring-spot formation on the inoculated leaves of *Datura* and a snake-skin-like mosaic pattern on the secondary leaves. The leaves remain on the plant and a mottled new growth develops. Tip-blight virus causes a general necrosis and, eventually, a complete defoliation of *Datura*. No new growth is formed as the terminal meristems are destroyed. A highly developed type of ring spot is the distinct feature of spotted-wilt virus on *Solanum capsicastrum*. The tip-blight virus produces a uniformly black, necrotic, local lesion on this plant. The 2 viruses develop a similar mottle on the nasturtium, but the necrotic lesions produced by the tip-blight virus are more regular in outline and darker in color. Both viruses form local lesions on Bliss Triumph potato, but the tip-blight lesion is more regular in outline and larger and shows progressive development. (Fig. 3, A and B).

The physical properties of the 2 viruses are similar, but certain differences can be noted. Although spotted wilt is difficult to transmit mechanically, it is possible to secure transfer with methods that have failed to transmit the tip-blight virus. The thermal death point of the tip-blight virus is between 40° and 41.5° C. as compared to 42° C. for spotted wilt. The spotted-wilt virus is more resistant to aging *in vitro*. It retains its activity for 5 to 6 hours at room temperature, while the tip-blight virus loses its activity in less than an hour under the same conditions. A dilution end point as high as 1–100,000 has been reported (17) for spotted wilt; tip-blight virus loses its activity when diluted 1 to 50. These properties tend to place spotted-wilt virus and tip-blight virus in a similar group, but they indicate also a distinct difference between the two.

DISCUSSION

These studies have demonstrated that the tip-blight virus has met the requirements used as a basis for the establishment of a "new" virus. Although this virus is known to occur only in Oregon, a critical review of the literature suggests that tip-blight virus is widely distributed but not recognized. Samuel, Bald, and Pittman (8) make the following statements in their descriptions of spotted wilt, "Very occasionally the whole plant dies, but only when affected at an early stage. Occasionally the growing point is killed while the lower part of the plant remains alive for weeks. But in the majority of cases affected plants are merely arrested in their growth, with only such necrosis of tissue as follows the more or less localized spotting on the upper leaves and stems." Kenneth M. Smith

(14) makes the following statement: "Occasionally tomato plants are killed outright by a severe necrosis resembling streak. This usually occurs when the plant has been infected as a young seedling; normally, however, the plant is not killed." These occasional symptom digressions may be explained as cases where both spotted-wilt virus and tip-blight virus were present. During the writer's experiments 80 tomato plants have been inoculated with a known pure strain of spotted-wilt virus. These inoculated plants were of various ages and sizes, but no plant developed any of the variations noted by the above workers.

Reports of spotted wilt in different parts of the United States also suggests a confusion of the 2 viruses. Blood's (1) description of an "unusual" occurrence of spotted wilt in Utah is more typical of tip blight than of spotted wilt. Shapovalov (11), (12), in his work with die-back streak, which he later states is identical to spotted wilt (13), gives descriptions that suggest a combination of spotted-wilt and tip-blight viruses. Young, Altstatt, and Harrison (19) report symptoms from Texas that suggest a mixture of tip-blight and spotted-wilt viruses. During these experiments both viruses have been found in the same locality, and, in some cases, both viruses were found on the same plant. It is conceivable that one, unacquainted with the characteristic behavior of the 2 viruses, might mistake the disease they produce in combination for a single virus effect. Since the tip-blight virus is the more sensitive to aging, and the more difficult of the two to transmit mechanically, the spotted-wilt virus is the only one usually recovered from doubly infected plants. The tip-blight virus predominated in pure form in Oregon, and the results were not confused by the presence of the spotted-wilt virus.

No method is known whereby tip-blight virus can be recovered from a mixture with spotted-wilt virus, but, as stated above, spotted-wilt virus can be recovered by mechanical inoculations or by aging the inoculum. If either virus is obtained separately its identity can be determined by inoculating tomato plants. If small tomato plants are killed within 2 weeks the virus is tip-blight; but, if the foliage becomes bronze-color and the growing tip becomes dwarfed, the virus is spotted wilt.

SUMMARY

Studies on the tip-blight virus of tomatoes are reported. The virus is described as new. A brief account is given of the symptoms produced by the virus on tomato plants. Some of the physical properties of the virus have been studied, and the virus has proved to be very sensitive. The thermal death point lies between 40° and 41.5° C., the longevity *in vitro* is less than an hour, and the dilution end point is less than 1 : 50. The virus is difficult to transfer mechanically. The virus is transmitted by *Thrips tabaci*. The symptoms produced by the virus on several differential host plants are described, and methods of identifying this virus are discussed.

OREGON EXPERIMENT STATION
CORVALLIS, OREGON

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STUDIES ON SOLANUM VIRUS 4

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Solanum virus 4—also known as potato virus B and as Up-to-Date streak virus—has been extensively studied by Clinch and Loughnane (3) and by Bawden (2), neither of whom was able to obtain it in a condition free from Solanum virus 1 (potato virus X). These workers were consequently unable to ascertain the symptoms of infection by Solanum virus 4 on solanaceous plants other than the potato, or to decide whether it alone could cause top necrosis on those potato varieties that are killed by Solanum virus 1, *i.e.*, on Epicure, King Edward and Arran Crest. In 1935, Dykstra (4) reported the discovery in American potato varieties of a virus apparently identical with "B" and stated that he had freed it from X by passage through the X-resistant potato seedling U.S.D.A. 41956. Beyond a statement that the "purified" streak virus was carried without symptoms by tomato and that it killed Arran Victory and President with top necrosis, he made no contribution to the study of its properties. By courtesy of the same worker a number of tubers of the seedling U.S.D.A. 41956 became available for use at the Potato Virus Research Station, Cambridge, and their immunity from Solanum virus 1 was confirmed by grafting and inoculating them with 6 strains of that virus. In no case did the X-resistant potato plant become infected, and it was accordingly decided to utilize this variety to obtain an X-free strain of Solanum virus 4, the properties and host range of which could then be studied in detail.

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[1] The writer has pleasure in expressing his thanks to Dr. K. M. Smith and Mr. F. C. Bawden for carrying out the filtration and serological investigations, respectively, and to Dr. R. N. Salaman for his unflagging interest and criticism throughout.

HOST RANGE

The source of Solanum virus 4, used in the present work, was a plant of Up-to-Date potato known to be carrying both the streak and a mild strain of Solanum virus 1 and believed to contain no other viruses. Presence of the streak was confirmed by grafting the Up-to-Date to healthy plants of President and Arran Victory, both of which succumbed to top necrosis. On May 24, grafts were made from the Up-to-Date to 2 plants of U.S.D.A. 41956, both of which apparently remained healthy throughout their growing period. On July 14 a scion from one of these plants was grafted to healthy *Datura stramonium,* which, some 3 weeks later, exhibited necrotic veinal etching of the older leaves, followed by bright mottling and crinkling of the new growth. Inoculation from the parent 41956 to other *Datura* and *Capsicum* plants failed to infect them, but the virus was found to be readily sap-transmitted from the tissues of the grafted *Datura.* Symptoms of infection by sap-inoculation to solanaceous plants may be summarized as follows:

(1) *Datura stramonium.* No local lesions occur, but the old leaves develop a conspicuous, white, etched network along the finer veins. This is seen on the inoculated leaf some 10 days after infection, and is followed in the case of young plants by systemic vein clearing with marginal shriveling and mass necrosis of the apical leaves. In larger plants there is less necrosis, and, about a month after inoculation, the upper leaves show a rather fine dendritic yellow mottle (Fig. 1, D) distributed chiefly along each side of the major veins and joining up, to leave large green islands of unaffected lamina. On the youngest leaves small necroses arise, and in old plants the leaves show only a bright yellow interveinal mottle with irregular dark green veinbanding, accompanied by small scattered necroses and by much curving and ruffling of the lamina. The plants never completely recover, although, several months after inoculation, there may be less necrosis and distortion of the leaves. Flowers and fruit appear normal.

(2) *Nicotiana tabacum* var. White Burley. There are no local lesions and the plants develop a rather bright regular systemic interveinal mottle of tortoise-shell type. Occasional light necrotic spots or rings may arise, especially in old plants.

(3) *Nicotiana sylvestris.* No local lesions. Systemic symptoms as on tobacco.

(4) *Nicotiana alata.* No local lesions and systemic symptoms develop very slowly. Some 2 months after inoculation a very bright yellow interveinal mottle arises on the young leaves and large yellow-green patches come to occupy most of that portion of the interveinal areas that lies next to the main vein and towards the base of the leaf.

(5) *Nicotiana glutinosa.* No local lesions. Systemic veinbanding mottle as on tobacco, but no necroses.

(6) *Lycopersicum esculentum* var. Kondine Red. No local lesions, but a very marked systemic veinal mottle that slowly extends into the interveinal areas and is accompanied by marked rugosity and waving of the lamina. About 6 weeks after inoculation this is replaced by a bright yellow interveinal blotch, leaving a dark green band along the main veins and this symptom persists almost unchanged throughout the life of the plant. Flowers and fruit appear normal.

(7) *Lycopersicum racemigerum.* Very similar to tomato, but small, dark brown, local lesions are produced.

(8) *Lycopersicum piriforme.* Like *L. racemigerum*, but there is a greater tendency toward development of light necrotic spots on the systemically infected leaves.

(9) *Capsicum annuum* var. Golden Dawn. Whitish, concentric rings arise on the inoculated leaves about one month after infection, and are quickly followed by interveinal mottle and necroses on the young leaves. In general, the symptoms are indistinguishable from those caused by a mild strain of Solanum virus 1.

(10) *Solanum nigrum.* Few small, brown, local lesions, followed by mild systemic interveinal mottle.

(11) *Solanum nodiflorum.* As with *S. nigrum*, but the systemic symptoms are more transient.

(12) *Nicandra physaloides.* Few small, dark brown, local lesions, followed by light, systemic, interveinal mottle with slight superficial necrosis and much ruffling of the lamina.

(13) *Hyoscyamus niger.* Round, white, papery, local lesions and very bright yellow, systemic, interveinal mottle, leaving a narrow dark green band along the vein of the leaf. Necrosis of the lamina very slight or absent.

(14) *Petunia nyctaginiglora.* No local lesions, but a rather conspicuous systemic interveinal mottle develops some 3 weeks after inoculation. The virus is readily recovered from the mottled leaves. Solanum virus 1 is not sap-inoculable to most strains of *Petunia* spp.

(15) *Salpiglossis variabilis.* No local lesions arise, but the young leaves develop a generalised interveinal mottle with small, pale brown, necrotic spots.

(16) *Browallia speciosa.* No local lesions, but a quite definite, systemic, interveinal mottle develops some 10 days after inoculation.

(17) *Atropa belladonna.* No symptoms were observed and the virus could not be recovered from the inoculated plants.

(18) *Lycium barbarum.* Small, brown, local rings developed on the inoculated leaves some 3 weeks after infection. No systemic symptoms were observed, nor could the virus be recovered by punching out the local lesions and inoculating from them to tobacco.

(19) *Solanum tuberosum.* Previous workers have shown that Solanum virus 4 is not sap-inoculable to most potato varieties and that it usually induces local lesions on the inoculated potato leaf. Clinch and Loughnane (3) reported it to be sap-inoculable to Arran Crest, which it killed by top necrosis, but this observation was explained by Bawden (2) as due to contamination with Solanum virus 1. The latter author, dealing with a mixture of Solanum viruses 1 and 4, found that following inoculation to potato, virus 4 was left in the local lesions, whilst virus 1 frequently passed from the inoculated leaf and systemically infected the plant. He could, however, readily transmit virus 4 to potato by grafting with a *Datura* scion infected with viruses 1 and 4. In the present work it has been found almost impossible to infect potatoes by use of *Datura* scions containing only virus 4. In such cases the scions die or produce roots that penetrate the hollow of the potato stem and nourish the *Datura* without true union taking place with the stock. This failure seems to be due to the extreme severity of the disease produced by Solanum virus 4 alone on *Datura* and has led to the employment of tomato plants as scions. The results of these grafts may be summarized as follows:

> Top necrosis developed with Solanum virus 4 alone on President, Arran Victory, British Queen, King Edward, Epicure, Arran Crest, Alpha, Katahdin, Doon Early, and Ballydoon. The virus was carried without symptoms by Up-to-Date, Majestic, and U.S.D.A. 41956, its presence in them being subsequently demonstrated by fresh grafts to Arran Victory. The rapidity of top necrosis varied with the variety. Arran Victory was extremely susceptible and its growing points were usually killed between 3 and 4 weeks after grafting; President seemed less susceptible, while such varieties as Katahdin, Doon Early and Alpha developed top necrosis very slowly and, in some cases, although the typical black interveinal necroses appeared on the apical leaves, the growing point was not killed before the plant matured. Bawden (2) has described a somewhat similar restricted top necrosis in the case of grafts made from *Datura* infected with Solanum viruses 1 and 4 to President carrying Solanum virus 1.

When Solanum virus 4 was sap-inoculated from tobacco or *Datura* to Arran Victory or President, large blackish local lesions appeared on the inoculated leaves, but no systemic infection resulted. No virus could be recovered from the top growth of such plants, though special search was made for Solanum virus 1, on the supposition that slight contamination with it might still remain. When similar sap inoculations were made to Arran Crest or King Edward, however, the plants died with top necrosis. Small local lesions developed on the inoculated leaves, and the first systemic symptoms were observed in the form of interveinal necroses on the top leaves some 4 weeks after inoculation. Top necrosis was usually not complete for another 4 to 6 weeks. Inoculation from the necrotic apical leaves of these potatoes to *Datura* gave the typical Solanum virus 4 picture, but the presence of the streak could not be confirmed by grafting these *Daturas* to Arran Victory or President, owing to the failure of the scions. That the top necrosis following inoculation of King Edward was caused by the Solanum virus 4 was shown by using tomato as the intermediate host.

On February 2 a plant of King Edward was inoculated from tobacco infected with the streak virus. The inoculation was made by sprinkling a little carborundum powder on the leaves and rubbing them lightly with a pestle dipped in the infective juice. The leaves were then rinsed under a tap to remove excess sap, as experience has shown that it is much easier to infect potatoes by sap inoculation when this precaution is taken. Ten days later small, round, local lesions appeared on the inoculated leaves, and, during the next week, they became confluent and spread along the vein, causing shrivelling of the rest of the lamina. Systemic symptoms appeared on March 11 as a sparse, yellow, veinal mottle of the upper leaves. Small blackish necroses, recognized as early top necrosis, were observed on the apical leaves on March 22, and, by April 11, the plant was nearly dead with top necrosis. On March 22 inoculation was made from the necrosed upper leaves to healthy tomato seedlings, which subsequently developed the characteristic bright yellow interveinal mottle. On April 11 a scion from one of the tomatoes was grafted to a healthy President, which died of top necrosis some 2 months later. It is, therefore, evident that the virus that became systemic followed inoculation to King Edward was Solanum virus 4 and not a contaminating strain of Solanum virus 1.

PHYSICAL PROPERTIES

The physical properties of Up-to-Date streak were studied by Bawden (2) who found that in a mixture of Solanum viruses 1 and 4 only the former remained after dilution of the inoculum to 1:1000 or after ageing for 4 weeks *in vitro*. Both were inactivated by heating infected sap for 10 minutes at 70° C., while neither was affected by heating for the same period at 60° C.

Working with infected tobacco sap, the writer has found the strain of Solanum virus 4 passed through U.S.D.A. 41956 to be inactivated by exposure to 70° C. for 10 minutes, but not by 65° C., for the same period. It was recovered after dilution to 1:100,000. It withstood storage *in vitro* at room temperature for 6 weeks, but not for 10 weeks. Filtration of a Kieselguhr filtrate of infected sap through gradocol membranes was kindly carried out by Dr. Kenneth Smith, who recovered the virus unchanged after passage through a membrane of average pore diameter 0.175 μ. It was not found in the filtrates from a membrane of pore size 0.057 μ. These properties closely resemble those of Solanum virus 1, which is, however, more resistant to ageing *in vitro*.

COMBINATION WITH OTHER VIRUSES

Some light may be thrown on the relationships of a plant virus by its reactions in combination with other viruses on a single host plant. The reaction of Solanum virus 4 has been tested in combination with Nicotiana virus 1 and with Solanum virus 2.

(a) Combination with Nicotiana Virus 1 (Tobacco Mosaic)

Three strains of this virus were used in *in vitro* mixtures with Solanum virus 4, viz., "Normal" Nicotiana virus 1, 1 C (tomato aucuba) and 1 D (Holmes' masked strain). The following descriptions apply particularly to the "Normal" strain—reactions with strains 1 C and 1 D were slightly more and less severe, respectively.

On tobacco the combination gave rise to whitish, papery, local lesions about ⅛ m. across, with darker margins. These were followed by severe necrotic systemic vein clearing, and, a fortnight after inoculation, the plants were severely stunted with mass necrosis of the lower halves of those leaves on which systemic symptoms first showed. After a further 14 days the plants were only about one third the size of those infected with Solanum virus 4 alone, and their leaves exhibited bright interveinal mottle with very numerous large. white, papery, necrotic spots. A month later they were all either dead or dying.

On tomato large grey lesions were observed, followed by numerous small systemic necroses. A fortnight after inoculation the plants showed a bright yellow interveinal mottle with interveinal necroses. A few leaves had been killed by mass necrosis and streaks spread down the stems from their axils. The plants, though stunted, were not killed outright and ultimately bore normal flowers.

It will be observed that both on tobacco and on tomato the symptoms are like those of the "streak" resulting from a combination of a severe necrotic strain of Solanum virus 1 with Nicotiana virus 1.

(b) Combination with Solanum Virus 2 (Potato Virus Y) on Tobacco

Lightly etched, ring-like, local lesions were observed, followed by bright systemic vein clearing. The latter rapidly became necrotic and led to some distortion of the leaves, but the plants were not killed. The symptoms closely resembled those of a severe infection with Solanum viruses 1 + 2.

PURITY OF THE VIRUS USED AND ITS RELATION TO SOLANUM VIRUS 1

The original Up-to-Date source, apparently, contained only Solanum viruses 1 + 4; consequently, the question arises, was all trace of 1 removed by passage through U.S.D.A. 41956? There are 2 lines of evidence that might be held to indicate that contamination of this kind had not been entirely eliminated. The first of these is the readiness with which varieties like Arran Crest and King Edward succumbed to top necrosis following sap inoculation with the streak virus. It has been shown, however, that the virus recovered from such a King Edward was capable of inducing top necrosis in President and was, therefore, not Solanum virus 1 alone; *i.e.*, if 1 and 4 were present together in the original inoculum then 4 was not left isolated in the local lesions, as described by Bawden (1) in the case of inoculation of such a mixture to other potato varieties. Moreover, attempts to recover a strain of Solanum virus 1 from previously healthy Arran Victory and President plants, after inoculation with the streak virus, were uniformly unsuccessful.

A more important indication of contamination with Solanum virus 1 is that given by the serological reaction of the streak. Mr. F. C. Bawden, who kindly tested the serological reaction of tobacco sap infected with the author's strain of Solanum virus 4, found it indistinguishable from Solanum virus 1.

Further light is shed on this subject by the degree of protection that infection with one of these viruses exerts against reinfection with the other. Bawden (1) has already shown that Solanum virus 1 in *Datura* exerts no protective effect against the streak and does not always prevent its entrance to potato. The writer has confirmed these observations. Thus, *Datura,* infected with a "masked" or symptomless strain of 1 and reinoculated 9 days later with 4, was not protected against the latter. Bright yellow spots appeared on the young leaves 4 weeks after reinoculation, and these were followed by a general bright interveinal mottle accompanied by necrotic specks, ruffling, and waving, almost as severe as in infection with Solanum virus 4 alone. When the preliminary infection was carried out with 1 G, a strain that causes a mild vein-binding mottle in *Datura,* reinoculation resulted in a rather bright yellow interveinal blotch only, without crinkling or necrosis. When the 1 L strain, itself characterized by bright yellowish interveinal mottle, was used, no change in symptoms followed reinoculation with Solanum virus 4. It should perhaps be pointed out that the protection afforded by infection with the mild H or G strains of Solanum virus 1 against reinfection with necrotic strains, such as S or N, is absolute (5).

It is similarly easy to demonstrate the absence of protection by Solanum virus 1 against Solanum virus 4 in potato. Plants of President systemically infected with strains 1 G, 1 L, and 1 S, respectively, were killed by top necrosis when grafted with tomato scions infected with the streak virus. Lack of protection by Solanum virus 4 in *Datura* against reinfection with Solanum virus 1 cannot be demonstrated because of the severe symptoms induced by the former alone. It has, however, been shown in tobacco by the following method. A set of 12 young tobaccos was inoculated with the streak virus, and, 9 days later, when all exhibited a mild interveinal mottle, 6 were reinoculated with the intensely necrotic N strain of Solanum virus 1. Eight days later, a few local lesions were observed on one of the reinoculated plants; and, after a further 10 days, the latter exhibited a seminecrotic yellow interveinal mottle with some lightly etched lines and rings. The

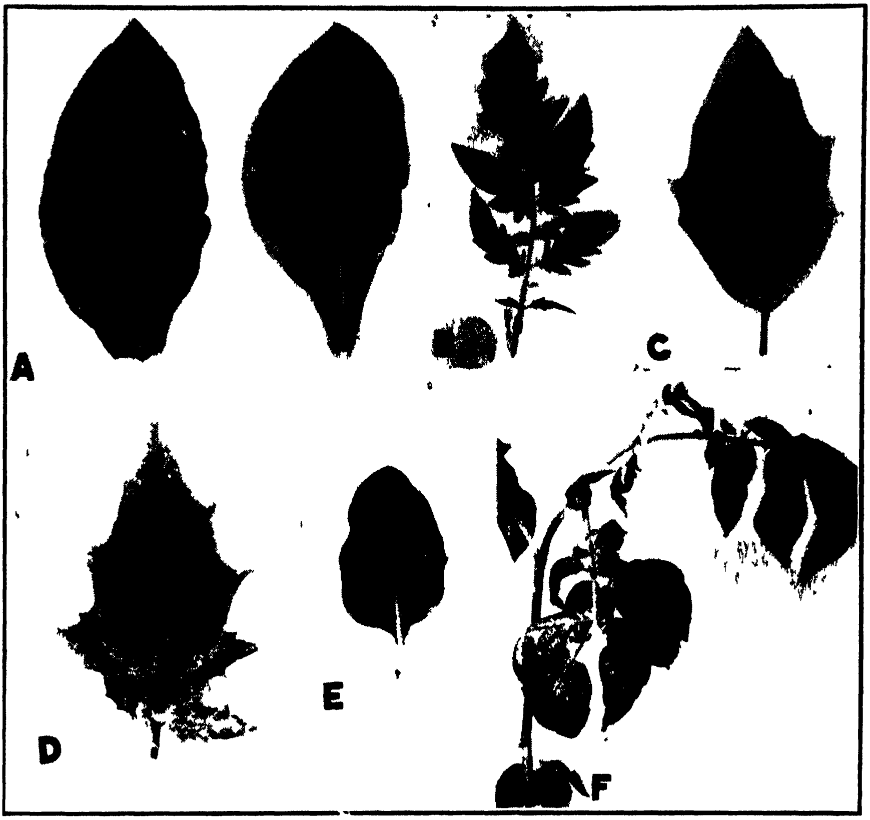

FIG. 1. A. Left: Solanum virus 4 on tobacco. Right: a similar plant after reinoculation with a necrotic strain of Solanum virus 1. B. Solanum virus 4 on tomato. C. Light necrotic etching caused by Solanum virus 4 on old leaves of *Datura stramonium*. D. Bright "dendritic" mottle caused by systemic infection of *Datura stramonium* with Solanum virus 4. E. Local lesions following inoculation of *Hyoscyamus niger* with Solanum virus 4. F. Early stage of top necrosis, in Arran Crest potato, resulting from inoculation with Solanum virus 4 after passage through U.S.D.A. 41956.

other reinoculated plants showed no local lesions, but developed numerous yellow spots, while the controls showed only a green interveinal mottle, as before.

In the case of potato it has been possible to demonstrate more clearly the absence of protection by Solanum virus 4 against Solanum virus 1. The variety Majestic carries the former virus without symptoms, and is remarkably sensitive to certain strains of the latter, including the strain originally described by Bawden (1) as the virus of Foliar necrosis (Virus "D").[2] A tomato scion infected with Up-to-Date streak virus, recovered from U.S.D.A. 41956 and filtered through a gradocol membrane of pore diameter

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[2] Foliar necrosis was originally described by Bawden as attributable to a distinct virus, which he called "D." It is now known to be caused by a strain of Solanum virus 1, distinguished mainly by its virulent reaction on potato. A potato plant infected with any other strain of Solanum virus 1 is completely immune from subsequent sap inoculation with foliar necrosis virus.

0.300 μ, was grafted to a healthy plant of Majestic potato. Good union between scion and stock resulted, but the latter remained apparently healthy. Three weeks later, however, the presence of virus 4 in the stock was demonstrated by grafts from it to a healthy plant of Arran Victory, which subsequently died of top necrosis. The Majestic plant was then sap-inoculated with the foliar necrosis strain of Solanum virus 1, and, 5 weeks later, it developed the symptoms of severe foliar necrosis. A control plant infected with streak virus, but not reinoculated, remained apparently healthy throughout.

In view of the above facts one seems justified in assuming that passage through U.S.D.A. 41956 has freed the Up-to-Date streak from contamination with Solanum virus 1 and that the symptoms described are due to the former virus alone. The close relationship between the two viruses is shown by their similar host range, their almost identical physical properties, and by the failure to distinguish between them serologically. So far as is known, they differ mainly in their reaction on certain potato varieties and in the failure of either to protect against reinfection with the other. It is on the latter fact alone that Solanum virus 4 can rest its claim to definitive status.

SUMMARY

Solanum virus 4 (B) was freed from contamination with Solanum virus 1 (X) by passage through the potato variety U.S.D.A. 41956. Its reactions, other than on the potato, have been studied on 18 hosts, of which *Datura stramonium* and *Lycopersicum esculentum* are found to be of the greatest value for diagnostic purposes. On the former the virus induces a bright systemic mottle with slight necrosis and deformity; on the latter a characteristic yellow interveinal mottle develops.

It has been found possible to infect King Edward and Arran Crest potatoes with Solanum virus 4 by sap inoculation, and the virus has been recovered unchanged from the former variety following such infection. In other potato varieties sap inoculation results in local lesion formation only.

Solanum virus 4 was inactivated at 70° C., but not at 65° C.; it endured dilution in tobacco sap to 1:100,000 and survived in expressed sap for 6 weeks.

Mixed infections of this virus with Nicotiana virus 1 and Solanum virus 2, respectively, confirm its affinity with Solanum virus 1.

Reinoculation experiments on tobacco, *Datura,* or potato show that previous infection of a host with either Solanum virus 1 or Solanum virus 4 does not prevent subsequent infection with the other virus.

It is concluded that the strain of Solanum virus 4 studied, was free from contamination with other viruses, and that the reactions described are, therefore, caused by it alone.

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LABORATORY STUDIES ON TOXICITY OF BENZOL VAPORS TO TOBACCO SEEDLINGS AND TO PERONOSPÔRA TABACINA[1]

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INTRODUCTION

Experiments recently conducted, both in Australia (1, 2, 3, 7, 10) and in the United States (9), have demonstrated that the vapors of benzol, C_6H_6, constitute an effective agency for the prevention and control of downy mildew of tobacco seedlings. Apparently, the difference between the concentration of benzol that is effective against the pathogen and that injurious to tobacco seedlings is sufficiently great to provide a wide margin of safety when benzol is employed as a gaseous fungicide. No quantitative data are available, however, as to the approximate minimal concentration of benzol vapors that is injurious to tobacco seedlings on the one hand or that is inhibitory or lethal to the downy mildew fungus on the other. The report here presented is, therefore, concerned with investigations conducted under controlled conditions to determine these limits of toxicity.

TOXICITY OF BENZOL VAPORS TO THE HOST

In Closed Systems

Preliminary information concerning the approximate limits of the concentration of benzol vapor injurious to tobacco plants was sought by exposing tobacco seedlings in bell jars or flasks. In both types of chambers, the pressures to which the seedlings were exposed slightly exceeded atmospheric pressure occasioned by the partial pressure of the benzol vapor present.

In these preliminary studies young seedlings, either of flue-cured or Burley varieties, potted in soil, were exposed to various concentrations of benzol vapor under bell jars (Fig. 1, A) sealed with various materials among which shellac proved most satisfactory. The required amount of liquid benzol[2]

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[1] Cooperative investigation conducted by the Virginia Agricultural Experiment Station and Duke University.

[2] Specifications for the commercial 90 per cent benzol used are as follows: Sp. gr. (15.5° C.) 0.882; mol. wt. taken as 78.05; visible color, No. 30 Saybolt; acid wash, No. 4; H_2S and SO_2, none; copper corrosion, negative; acidity, no free acid; sulphur, 0.14 per

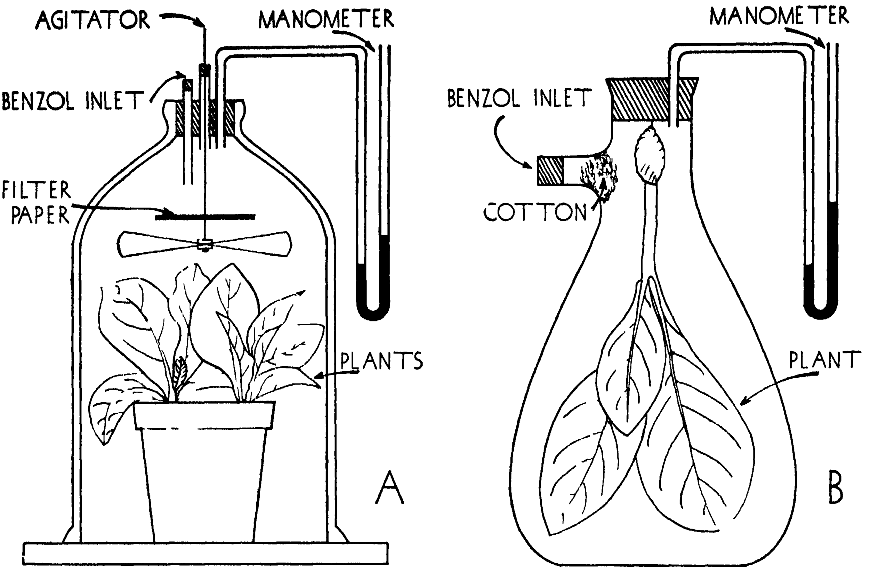

FIG. 1. Exposure chambers. A. Bell jar type with potted seedlings, benzol admitted through inlet, volatilization from filter paper aided by mechanical agitation. B. Suction-flask type, with suspended seedlings, benzol admitted by side arm, absorbed on cotton plug, volatilization aided by tilting the flask.

(commercial, 90 per cent) was introduced onto a strip of filter paper in the top of the jar by means of a pipette inserted through a convenient inlet. Thorough mixing of benzol vapor with air in the jar was effected by mechanical rotation of a paper agitator. In similar experiments, in which suction flasks (Fig. 1, B) were substituted for bell jars, inverted seedlings were suspended from the corks after the roots had been wrapped in moist cotton encased in tin foil. The flasks were then sealed and the desired amounts of C. P. benzol[3] introduced through the side arm onto a bit of absorbent cotton. Rotation of the suspended seedlings caused mixing of the enclosed vapors.

The calculated volume percentage[4] of benzol employed were 0.0, 1.0, 1.5, 2.0, 2.5, 3.0, and 5.0. Tests with each concentration were repeated several

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cent; toluol content, 4.0 per cent; distillation of first drop, 79.8° C.; 50 per cent, 81° to 82° C.; 90 per cent, 84° to 86° C.; dry 99.7° C. (See also footnote 4.)

[3] Sp. gr., 0.878 at 25° C.; mol. wt., 78.05.

[4] In this work all benzol vapor concentrations are expressed in terms of gaseous volume percentages according to the following formulae:

Volume per cent equals $\frac{p}{P} \times 100$ where p is the partial pressure of benzol vapor. Since the total pressure P differed but slightly from the normal barometric pressure of 760 in all calculations, P was regarded as equal to 760 mm.; hence, volume per cent equals $\frac{p}{760} \times 100$. From the gas law $\frac{pV}{760} = \frac{WRT}{M}$.

Where V equals the total volume of gases in ml.; T the absolute temperature; M the molecular weight (considered as that of benzol C_6H_6); W the weight in grams of benzol; R the gas constant (82.07 ml. atmos.) and p the partial pressure in mm. of benzol vapor from the volatilization of W grams of C_6H_6. 90% benzol was considered for the purpose of these calculations as C. P. material.

times. Periods of exposure of seedlings in these devices ranged from 12 to 19 hours. The temperatures ranged from 23.5 to 26.1° C.

Results of the above preliminary experiments indicated that under the conditions employed, concentrations of benzol vapor of less than 1.0 volume per cent did not injure the tobacco seedlings. Commercial benzol was harmless at 1.0 per cent by volume concentration in all cases, whereas injury was occasioned in one experiment with this concentration of C. P. benzol, with no injury, however, in another. In all experiments, concentrations of benzol vapor greater than 1.0 per cent by volume proved to be toxic. Since the seedlings always drooped after their removal from the chambers, judgment as to whether they had been injured during treatment was withheld until sufficient time had elapsed to permit them to recover their turgidity. If, after an hour or two, they appeared to be normal they were regarded as uninjured. If they remained flaccid and necrosis began to develop, the concentration of benzol vapor was considered toxic. Leaf tips and margins seemed to be more susceptible to injury than other portions of the leaf, and bud leaves most resistant.

In Chambers Permitting Gas Flow

As a result of the preliminary trials with tobacco seedlings exposed to benzol vapor in closed chambers, it seemed desirable to devise a means of continuously renewing the benzol-air mixture to which the seedlings were to be subjected. It was anticipated that this manner of exposure would more nearly approximate conditions of treatment existing in seed beds and would avoid any possible effects of the slight pressures resulting from the vaporization of benzol in the closed systems.

The apparatus devised for this purpose is shown in figure 2. It consisted of several large bottles and a circulation pump[5] so arranged in series that by connecting units B and G through pump K to form a closed system, and by introducing the required quantities of benzol through suitable ports in container F or pump K, the vapors could be mixed by repeated recirculation through all containers. Since the capacity of the pump was such as to circulate 360 l. of air per hour, mixing was considered complete after 3 hours of recirculation.

Upon removing the pump from the circuit and by connecting water reservoir A to bottle B and by connecting outlet G through sample flask H, the vapors were directed through the chambers I. All chambers were connected in series by means of a metal manifold. In order to displace the air initially in the chambers, 15 l. of the benzol-air mixture were forced through rapidly at the beginning of each experiment. The flow was then reduced to a standardized rate and time of 2.5 l. per hr. for 10 hours.

In a number of experiments, the results of which are presented in series 1 to 9, table 2, seedlings of the White Orinoco variety, 4 to 6 inches high, potted in sand, were enclosed in the chambers and held at temperatures of 21.1° to 26.6° C. In the remaining series seedlings of the Yellow Mammoth

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[5] An A. C. automobile fuel pump was found to be suitable for this purpose.

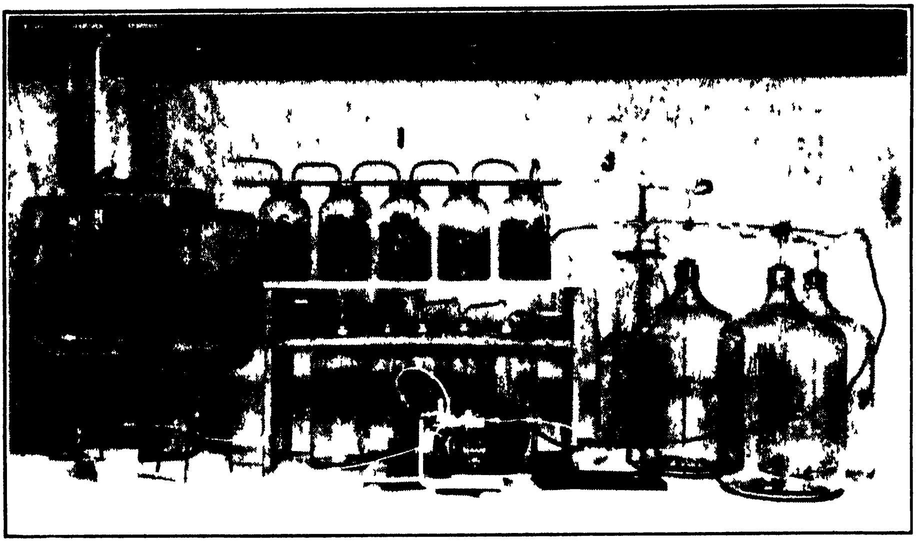

FIG. 2. Apparatus used for mixing benzol vapors with air and for medication. Water from reservoir A forced mixed gases from the system through plant chambers I. Absorption of benzol vapor by water in chambers B and C was minimized by the maze D and storage containers E, F, and G. Benzol introduced into sediment bulb of pump K or container F was mixed with the air in the system by circulation for approximately 3 hours through pump K. Manometer J indicated pressures within the system. The direction of gas flow through the system was from left to right in the following order: B, C, D, E, F, G, H, and I. Samples of the gas for chemical analysis were taken from flask H.

variety were grown in the chambers in 700 g. of steamed soil, held at two-thirds of its water holding capacity. An air thermostat with forced-air circulation was used to obtain temperatures of 8.1° to 10.0° C. To minimize the possible dilution of the gaseous mixture by absorption, all surfaces exposed to the vapors of benzol were insoluble or were protected with collodion or shellac. Stopcocks were lubricated with colloidal graphite in water to render them gas-tight. The glass maze D and storage containers E, F, and G served to minimize absorption of vapors in the water of containers B and C.

As a check to determine if the calculated quantities of benzol were actually present in the above mentioned apparatus, chemical determinations of the benzol content of the gaseous mixtures were made in series 3 to 9, table 2, by adaptation of a combustion method of gas analysis (8). By this method the benzol in benzol-air mixtures is converted to CO_2 and H_2O by passing the mixture through or over a tightly rolled piece of platinum gauze, 2 in. long. The platinum gauze was contained in a silica tube 12 in. long and $\frac{3}{8}$ in. in internal diameter. A piece of rolled copper gauze was placed in the receiving end of the silica tube as a flash-back arrester, thus preventing the spread of the reaction to the flask containing the benzol-air mixture. The platinum gauze was heated to a bright red heat by means of wing-tipped Bunsen burners. Contact of benzol vapor with the rubber connections was minimized by having the ends of connecting glass and silica tubes ground and of uniform size. As a further precaution a close-fitting glass tube was inserted inside the glass and silica tubes at the point where these tubes connected. In preparing the samples to be analyzed a weighed quantity of C. P. benzol, contained in a tarred bulb, was placed inside a 3800-ml. bottle. The bottle was closed with the outlet and inlet tubes in place, and the bulb broken by shaking the bottle. Shaking was resumed at intervals for about 30 minutes to aid mixing of the benzol and air within the bottle. By means of an aspirator, the benzol-air mixture being tested was drawn into the combustion chamber at a rate sufficient to utilize 40 l. of CO_2 free air in 1 to 3 hours. To insure a sufficient quantity of oxygen to completely convert the benzol to CO_2, a stream of carbon dioxide-free air was passed into the combustion tube along with the benzol-air mixture, if the benzol content of the mixture was greater than .04 g. per l. The CO_2 formed was drawn into a Milligan gas washing bottle containing 25 ml. of 2 N NaOH and 150 ml. of water. The excess NaOH was titrated with N/10 H_2SO_4, phenolphthalein serving as an indicator. The weight of CO_2 and C_6H_6 was calculated from these values.

Before presenting the results of experimentation on the limits of toxicity of benzol vapor to tobacco seedlings in open chambers, it appears advisable to indicate first the accuracy of the calculated volume-percentage concentrations used in the apparatus, as determined by the combustion method, and then to assemble the data on the reaction of the seedlings exposed in these chambers.

The data assembled in table 1 are taken from a body of analytical measurements secured by the combustion method and are given only to indicate its accuracy.

TABLE 1.—*Analysis of benzol content of benzol air mixtures*

Grams of C. P. benzol used	Grams of benzol calculated from the CO_2 formed	Percentage recovery
0.1330	0.1316	98.95
0.1354	0.1347	99.48
0.1412	0.1400	99.15
0.1550	0.1530	98.71

It is apparent from table 1 that the figures obtained by the combustion method of analysis vary less than 2 per cent from the calculated values of benzol employed.

Results relative to the effect of exposure of tobacco seedlings in chambers permitting gas flow are assembled in table 2.

An examination of the data obtained from exposure of the host plant in chambers at atmospheric pressures indicates that young tobacco seedlings are able to withstand appreciable concentrations of benzol vapors. Plants lacking any visible film of water on the foliage and maintained at temperatures of 21.1 to 26.6° C. were killed at vapor concentrations of between 3.0 and 4.0 volumes per cent. Plants covered with a film of moisture, at temperatures of 8.1 and 10.0° C., were killed at vapor concentrations of between 2.0 and 3.0 volumes per cent. This difference in toxic concentrations is undoubtedly caused by a combination of temperature and moisture effects. Owing to the

TABLE 2.—*Effect on tobacco seedlings resulting from a continuous flow of different concentrations of benzol vapor*[a] *for ten hours at atmospheric pressure*

Series	Chambers used	Benzol		Temperature of medication chambers	Condition of leaf surface	Remarks
		Calculated	Found by chemical analysis			
No.	*No.*	*Vol. %*	*Vol. %*	° *C.*		
1	5	0.0		26.6	Dry	No effect on seedlings
2	5	0.6		26.1	Dry	No effect on seedlings
3	5	2.0	1.906	24.5	Dry	Plants wilted on removal, soon recovered
4	5	2.5	2.439	25.8	Dry	Severe wilting, water-soaked areas, but no permanent injury
5	5	2.5	2.437	21.1	Dry	Severe wilting, no permanent injury
6	5	3.0	2.49	25.0	Dry	Severe wilting, no permanent injury
7	5	3.0	2.68	24.7	Dry	Severe wilting, no permanent injury
8	5	3.0	2.82	24.7	Dry	Moderate injury, necrotic areas
9	5		4.07	24.7	Dry	Plants were killed
10	6	0.0		10.0	Wet	No effect on seedlings
11	6	0.1		10.0	Wet	No effect on seedlings
12	6	1.0		8.9	Wet	No effect on seedlings
13	6	1.49		10.0	Wet	Very slight injury, necrotic areas
14	6	2.32		8.1	Wet	Serious injury
15	6	3.05		8.6	Wet	Plants were killed

[a] C. P. benzol was used in all series except No. 6 in which case 90 per cent benzol was employed.

solubility of benzol vapor in water, the latter is suspected of being the more significant.

Slight toxicity, characterized by severe wilting of the plants after removal from the plant chambers, was noted at lower concentrations. Plants subjected to vapor concentrations of less than 0.5 per cent by volume wilted slightly upon removal from the chambers, but recovered turgidity after 15 to 30 minutes. Wilting was more pronounced as the vapor concentration of benzol was increased, and longer periods of recovery were required. As the threshold of toxic action was approached, the leaves were very flaccid and tended to become a dark green. Plants in this condition recovered completely within 10 or 12 hours, and continued to grow normally when returned to the greenhouse benches. Plants subjected to lethal vapor concentrations appeared turgid up to the time of their removal from the plant chambers. The leaves were dark green and uniformly flecked with minute specks that appeared dark in reflected light and water-soaked in transmitted light. Within a few moments, upon removal from the plant chambers, the plants became flaccid, the cell sap seemed to exude to the surface, the color of the leaves became very dark green and the leaves completely collapsed, having an appearance somewhat resembling frost injury.

TOXICITY OF BENZOL VAPORS TO THE PARASITE

These studies were designed to obtain preliminary information on the concentration of benzol toxic to *Peronospora tabacina.*

For the first experiment 24 half-gallon jars in which tobacco seedlings were growing served as exposure chambers. The seedlings were inoculated by applying freshly formed sporangia to the moist leaves. Exposure to benzol vapors was effected by connecting 6 of the chambers containing the inoculated seedlings, with the apparatus shown in figure 2. These chambers were maintained at 9.5° C and exposed to 0.1 per cent by volume of benzol for 10 hours. The benzol vapor was forced through at the rate of 2.5 l. per hr. After the period of exposure, the 6 chambers were aerated, stoppered with sterilized cotton plugs, and set aside. Seedlings in 12 of the remaining chambers served as controls. After incubation for 7 days the seedlings in the 12 control jars had become severely infected and all succumbed a few days later, whereas no infection developed on the treated seedlings. In the case of the 6 remaining jars, maintained at temperatures favorable for infection, slight sporulation was apparent 5 days after inoculation. They were then exposed to 1.0 per cent by volume of benzol vapor for 10 hours at a maintained temperature of 9° C. As before, the rate of flow of benzol vapor was 2.5 l. per hr. After the period of exposure the plant chambers were aerated, stoppered with sterilized cotton, and set aside. Sporulation did not occur on these seedlings for 3 days following exposure to the vapor but was resumed on the fourth day, and after a few days the pathogen had killed all of the plants.

In another series of trials, clumps of infected seedlings were carefully uprooted and then transplanted into tin cups early in the morning. The tin cups were then placed in 1 gal. tin containers that had previously been lined with moistened towels. An opening in the lid of each container permitted the introduction of the calculated amounts of benzol, which were absorbed by filter paper fastened beneath the opening. A piece of cardboard, also fastened to the lid, served as an agitator when the container was rotated. After introduction of the benzol the openings were stoppered with corks. Concentrations of benzol of 0.1, 0.5, 1.0, and 2.0 per cent by volume were employed. Temperatures ranging from 20° to 24° C. were maintained. Three applications of benzol were made, the first of 16 hours' duration and that of the next two, 12 hours each. Nontreated infected seedlings served as controls.

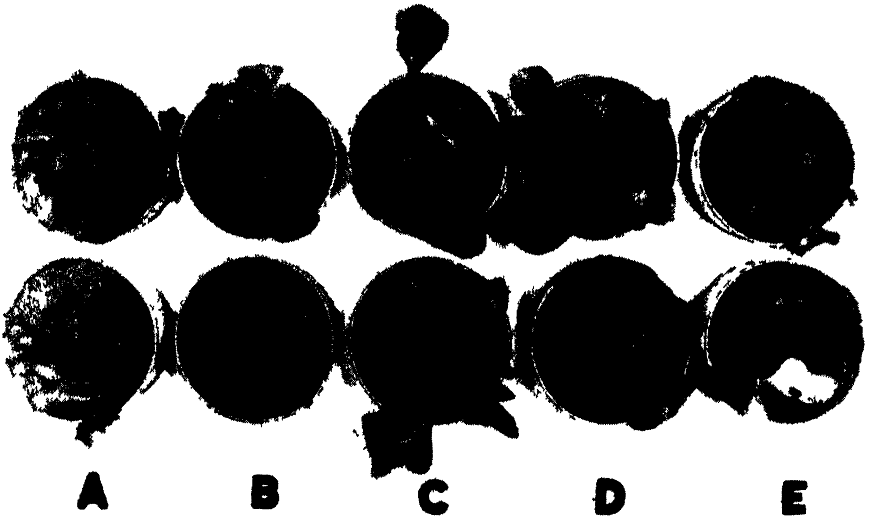

FIG. 3. Effect of different volume percentage concentrations of benzol in control of downy mildew on tobacco seedlings. Condition of seedlings 7 days after 3 consecutive treatments. Non-treated control, A; 0.1 per cent, B; 0.5 per cent, C; 1.0 per cent D; and 2 0 per cent, E.

Some appreciation of the effectiveness of this method of application of benzol vapors in the different volume percentages can be seen in figure 3. There were no living seedlings among the controls (Fig. 3, A) 7 days after the first treatment. By this time many of the seedlings subjected to consecutive exposures of 0.1 volume per cent of benzol vapor were dead, having been killed by the fungus, and others only barely survived (Fig. 3, B). Seedlings exposed to 0.5 (Fig. 3, C) and 1.0 volume per cent (Fig. 3, D) of benzol vapor had survived, new growth had been initiated, and recovery was soon complete. All seedlings subjected to a benzol concentration of 2.0 per cent by volume (Fig. 3, E) were severely injured by the combined action of the benzol and the fungus and eventually all died.

Further information was obtained by attempting to germinate the sporangia from the above series. At the close of the first period of exposure, sporangia from plants in each concentration of benzol vapor were placed in drops of water on microscope slides. After 22 hours 10 to 13 per cent of the sporangia from the series held at 0.1 per cent by volume of benzol ~~vapor~~ had germinated; 6 to 15 per cent, from the series held at 0.5 per cent by volume; none, from the series held at 1.0 or 2.0 per cent by volume; and 61 to 69 per cent, from the nontreated control series. Examination of the sporangia exposed either to 1.0 or 2.0 per cent by volume benzol revealed that they had been plasmolyzed.

DISCUSSION

The use of gaseous substances as germicides and disinfectants has been generally unsatisfactory, although entomologists have been successful in the use of certain gaseous insecticides. Plant pathologists have employed aqueous solutions as fungicides since Bordeaux mixture was first found to be effective against downy mildew of grape. More recently dusts have been used as protectants against certain fungi pathogenic to plants. Little attention has been directed, however, by plant pathologists toward the possibilities presented by gaseous fungicides, as was noted in a previous report (9). The use of benzol vapor as a protectant against downy mildew of tobacco marks the first instance of the successful use of a fungicidal gas upon growing crops.

It is apparent to all who have used benzol as a fungicide against tobacco downy mildew that higher concentrations are required to cause injury to tobacco seedlings than are necessary to inhibit the development of the pathogen or to be lethal to it. This observation is verified under the conditions of the present experiments, in which the approximate toxic limits of benzol-air mixture for both the host and the parasite has been determined. The lesser concentrations required to be toxic in chambers where the vapor pressures exceeded atmospheric pressure may perhaps be related to an increase in penetration by benzol vapors when the leaf tissues are under higher total pressures.

For several reasons these laboratory results might not be found to apply directly to conditions in tobacco seed beds. In the first place, one could not expect to maintain high concentrations of benzol vapor in seed beds, since the beds are not gas-tight. Secondly, it seems safe to assume that duration of exposure of both host and parasite to benzol vapors is a potent factor in conditioning the limits of toxicity. These factors make it unlikely, except during unusual weather, that one could expect continuously to maintain lethal or toxic concentrations of benzol in the atmosphere of seed beds for periods comparable with those to which the host and parasite were subjected in the chambers.

Particular emphasis should be placed on the observation that seedlings having no appreciable film of moisture on the leaf surfaces tolerated higher concentrations of benzol vapor than those covered with a film of water. This observation seems to be significant, under usual conditions of fumigation in seed beds, in the light of the fact that about 1.85 grams of benzol are soluble in one liter of water at 30° C. (5), an amount that corresponds to approximately 12.6 per cent by volume at 25° C. at atmospheric pressure in the gaseous state. Depending mainly upon time and temperature, benzol vapor would, therefore, continuously enter into solution in any water present whether on the leaves, within the leaf tissues, in the soil, or on the seed-bed covers. In any of these situations the volume per cent of gaseous benzol to which these solutions would correspond might be greater at any given moment than that in the atmosphere within the seed bed.

The toxicity of benzol to man and to laboratory animals has apparently been extensively investigated, as shown by the summaries by Ellinger (4) and Hamilton (6). Among the pathological changes induced by benzol poisoning in animals is a tendency to extravasation of blood. This phenomenon is manifest by bleeding either in small petechiae or large echymoses, the severity being related to the severity of the poisoning. Areas of bleeding may occur over the entire body or involve various internal organs as the lungs, kidneys, spleen, and liver. The number of erythrocytes and leucocytes and amount of hemoglobin are reduced. The kidneys, liver, heart, lungs, bone marrow, etc., are involved in fatty degeneration. These pathological conditions, among the numerous changes induced by benzol, all indicate that this hydrocarbon modifies the lipoidal constituents and permeability of animal cells.

Although much is known regarding the effects of benzol on animals, little is known of its effects on plants The mechanism of this toxic action, whether to animals or to plants, may reasonably be expected to have certain features in common. Once the cell walls have been wetted with aqueous solutions of benzol, in the case either of the parasite or of the host, the benzol presumably acts upon the lipoidal constituents of the plasma membrane, altering the permeability of the cells and their ability to function normally. This pattern of events apparently represents the mechanism of toxicity to plant cells, and appears to agree with that employed to explain pathological changes induced in animal cells.

Benzol differs in an important feature from fungicides generally, because it acts not only as a surface protectant but in addition appears to be lethal to the mycelium within the interior of the leaf tissues. It, therefore, acts both externally and internally to the tissues of the host plant.

SUMMARY

Attempts have been made by laboratory methods to determine the minimal concentrations of benzol vapor in air that are injurious to tobacco seedlings and the concentrations toxic to *Peronospora tabacina.*

Apparatus has been designed for use in treatment with benzol. A combustion method of analysis has been devised that is adapted for determination of benzol in benzol-air mixtures.

Among the several factors that appear to be involved in determining limits of toxicity are duration of exposure to treatment, number of applications, and presence of visible moisture on the foliage.

At atmospheric pressure, concentrations of benzol vapor in air greater than approximately 2.0 per cent by volume were injurious to tobacco seedlings if the foliage was wet during the period of medication, and those greater than 3.0, caused injury, if there was no visible moisture on the leaves. Concentrations of benzol vapor in air of approximately 0.5 per cent by volume or greater were lethal to sporangia of the tobacco-downy-mildew fungus. Repeated exposure of infected seedlings to less than 0.5 per cent by volume of benzol inhibited sporulation. Lower concentrations of benzol vapor were required to cause injury at pressures less than atmospheric pressure.

It is suggested that the mechanism of toxic action by benzol involves, first, absorption by the cell walls and then the dissolution of lipoidal materials in the plasma membrane with consequent adverse modification of permeability and correlated functions.

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FREEZING INJURY TO CANNING PEAS

J. C. WALKER

(Accepted for publication Sept. 30, 1938)

Extensive acreages of garden pea, *Pisum sativum* L., grown for canning in the upper Mississippi Valley, are often subject to freezing injury in April and May. Considerable damage occurred to the crop in May, 1930, and again in the same month in 1938. During the latter year the writer made certain observations and records of the morphological and physiological responses of the plant to freezing, which are presented in this paper.

NATURE OF THE FREEZING DAMAGE

Inasmuch as the greater portion of freezing damage is that that is sustained in the acreages grown for canning, the nature of the economic loss is discussed from that point of view. Since the crop is sown beginning in early April and continuing to mid-May, plants are exposed from mid-April on. By mid-May the earliest plantings are 6 inches or more in height. The most damaging frosts are those that occur at this time, since they usually follow a period of warm weather during which succulent growth has proceeded rapidly. Pea plants are seldom killed during such periods of freezing. If the temperature is low and uniform enough to kill all tops back to the surface of the soil, new growth usually will develop quite uniformly from protected subterranean nodes. In such cases the damage consists chiefly of delay in maturity. Early frosts often cause such injury and are relatively harmless compared with late frosts. The latter usually consist of temperatures that are only 2 to 4 degrees below 0 C. for short periods. The damage to plants is then marked by the wide range of severity between individuals, particularly in fields most advanced in growth. The spread in maturity in a single field is responsible for the greatest economic loss.

Inasmuch as successful pea canning rests on the culture of varieties that normally produce several pods at the prime canning stage within one or two days of each other, any environal factor, such as freezing injury, which upsets this compactness of maturity, is a seriously damaging one. This can be illustrated best by referring to figure 1 A. The two plants are representative ones from a field of early-planted Alaska peas, subjected to the frosts that occurred in mid-May, 1938. Since the details of disturbed morphology will be discussed later, it is sufficient to point out here certain points with regard to maturity. The plant at the left was only slightly damaged at the point marked by the arrow, but growth was not impeded and the plant had developed 3 pods at the canning stage when the sample was taken 6 weeks later. The uninjured plants in the field were at essentially the same stage. In this particular field more than 25 per cent of the plants were similar to the one at the right, which was injured with sufficient

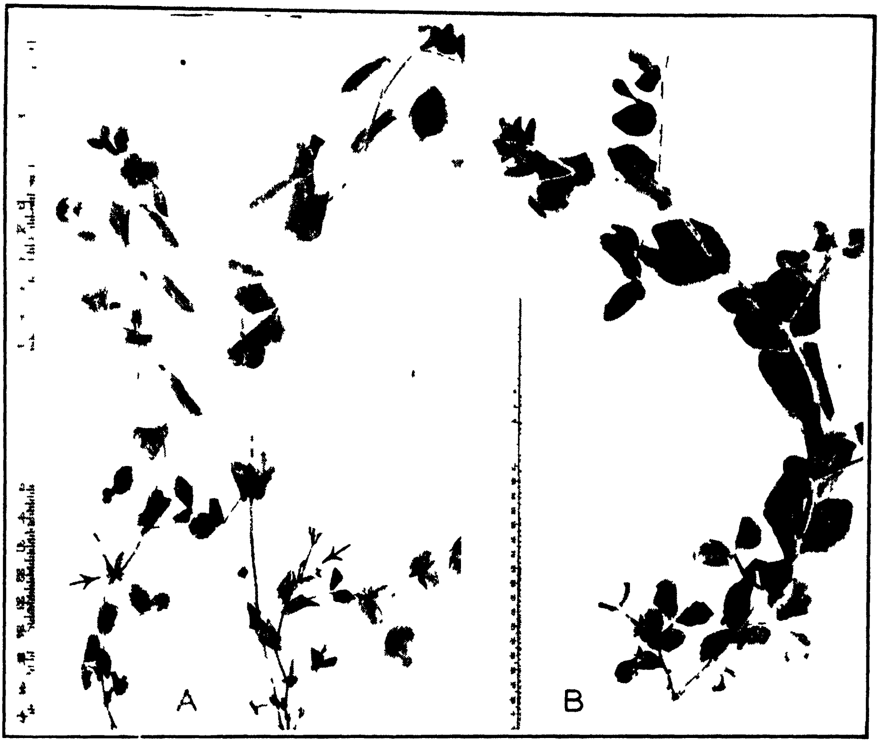

FIG. 1. Freezing injury to canning peas as revealed 6 weeks after the damage. Plants from a field near Madison, Wisconsin, which was subjected to freezing on May 11, 12, and 13, 1938. A. Alaska peas, which were about 6 inches above ground with the leaves at about the 9th node, unfolding at the time of the freeze. The growing point at this node was not killed in the plant at the left, although the leaflets were injured and malformed (arrow). In the plant at the right the leaflets also were injured (see arrow) and the growing point was killed. This resulted in new shoots from two lower nodes, one of which gained ascendency and produced pods. Note the characteristic tendency of this delayed shoot to become taller due to longer internodes. The unretarded plant has 3 pods at canning stage, while none have reached that point in the damaged plant. B. Plant of Perfection variety, which, having been planted later, had not grown so far as the Alaska plants in A at the time of the freeze. The growing point was killed at the 7th node and one new shoot developed at the 2nd node. (See enlargements of injured regions in Fig. 2.) In this and in the other figures the smallest subdivisions in the scale are millimeters.

severity at the point indicated by the arrow to kill the growing point. Stimulation of lower buds to growth and expansion resulted in the prompt development of a new main stem on which 3 pods also had developed at the time the samples were taken. Due to the delayed growth, however, the seeds in the pods of the badly injured pods were not sufficiently mature for canning. Such plants become a total loss because of the fact that the entire field must be harvested in one operation, and the time of cutting is determined by the date at which a majority of the plants reach the canning stage.

The second major source of damage due to freezing of peas is in connection with the seed production, especially of canning varieties. Although the disturbance in maturity is not important in this culture, insofar as yield is concerned, the irregularity in growth of plants seriously interferes with and often entirely prevents the important procedure of roguing, which consists of detection and elimination of off-type plants during the growing season.

PLANT RESPONSE TO FREEZING INJURY

The effects of freezing injury may be distinguished clearly a few days after the damage occurs. The most characteristic symptoms, however, are those that develop after a somewhat longer interval, since they result from abnormal growth of injured leaves and stems. These signs are particularly useful in distinguishing freezing injury from other maladies, as well as in estimating the extent of loss resulting from the former.

The growing point of the main stem and the most recently formed leaves that commonly surround it contain the most susceptible tissue. When the growing tip is killed, the prompt physiological response of the plant is the stimulation of one or more dormant buds at the leaf axils along the main stem. The older nodes nearer the soil line usually respond but this is not always the case. One or more leading stems may thus develop, one of these often taking precedence (Fig. 1). When a single new stem gains ascendency it commonly overtakes the uninjured plant in length because of the abnormal extension of internodes. Thus, as a field that has been frosted

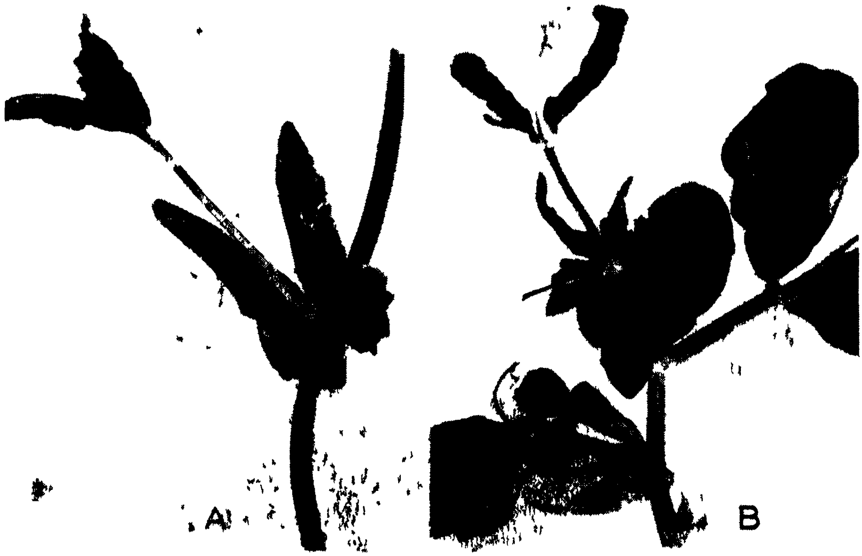

FIG. 2. A. Enlargement of injured node in the plant at the left in figure 1, A. The leaf was injured so that only two malformed leaflets developed, while the stipules were abnormally long and narrow. The growing point developed normally. B. The injured node in the plant shown in figure 1, B. The growing point was killed and the stipules became malformed (see arrow). The leaf at this node consisted of the first two leaflets that were distorted, while the second pair never formed and the tendril was reduced in size.

approaches the canning stage, the frozen plants can often be distinguished by the fact that they are taller and protrude above the others. This is shown best by comparison of the two plants in figure 1, A. It is this type of irregularity that interferes with effective roguing.

The youngest leaf and its stipules continue to grow, though damaged. Certain of the features are shown in figure 2, which consists of enlargements of the injured portions of two of the plants in figure 1. The only evidence of frost damage to the plant at the left in figure 1, A, is the

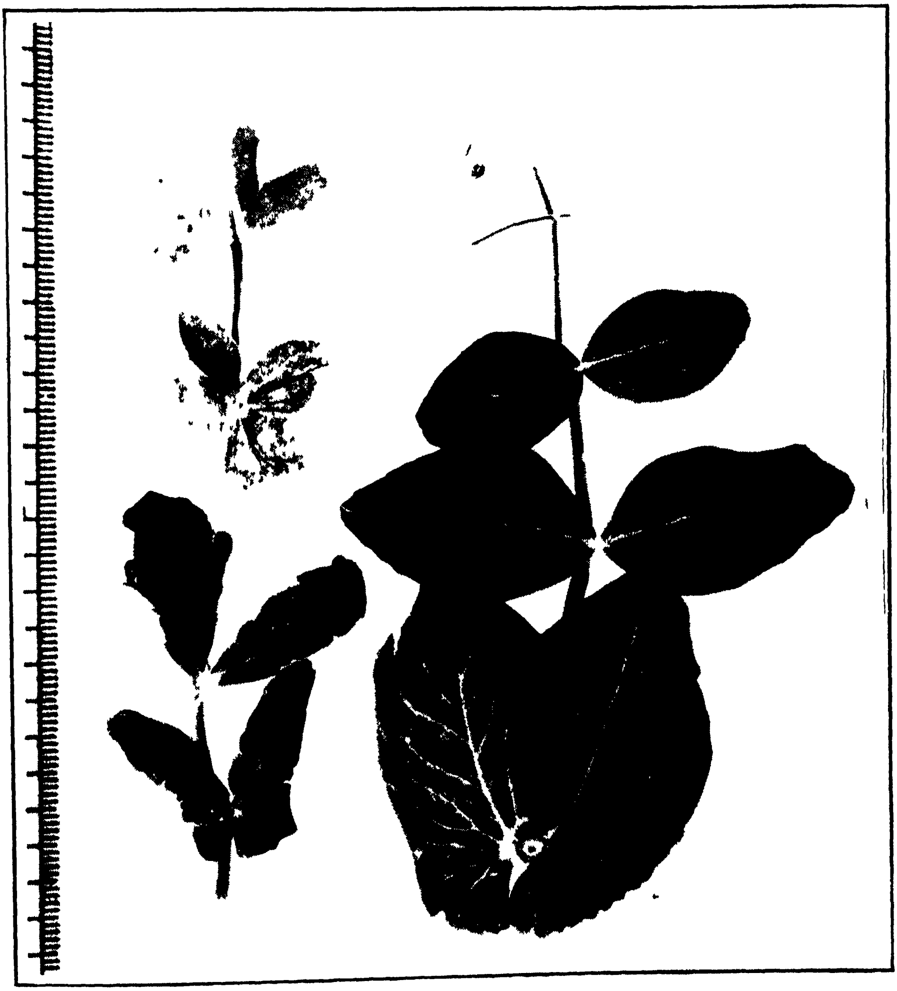

FIG. 3. Leaf malformation following freezing injury. The injured leaves at the left are of the same age and have developed under the same environment as the uninjured leaf at the right. Complete killing of the buds of the second pair of leaflets is common, while normal tendril expansion is reduced in various degrees. Sepals and leaflets, besides becoming stunted, expanded abnormally, usually becoming more elongate, as in the lower leaf, and sometimes assuming a bilobed form, as in the upper leaf. Specimens collected 6 weeks after injury occurred.

morphology of the injured leaf shown in more detail in figure 2, A. In this case the growing point of the stem was not killed and normal stem expansion occurred, but the embryonic leaf enclosing the meristematic tissue was injured. In figure 2, B, the remains of the killed meristem are indicated by the arrow. A study of the injured leaves in figure 2, A and B, and in figure 3 reveals the major effect of freezing on the embryonic organs. The stipules, besides being reduced in size, are abnormal in shape. They are usually relatively long and narrow, while the margins tend to be wavy and irregular in contour. The first pair of leaflets usually develops, while the second pair is almost always suppressed. The same tendency toward elongate expansion and irregular wavy margins as noted in the sepals prevails. The tendril is also suppressed or retarded in varying degrees. A less common morphological response of leaflets and sepals is that shown in the upper leaf in figure 3. The leaflet or sepal becomes equally or unevenly divided and expands to form two distinct lobes.

Equally characteristic is the response of leaves and sepals that are slightly older at the time of injury. The gross morphology is then not

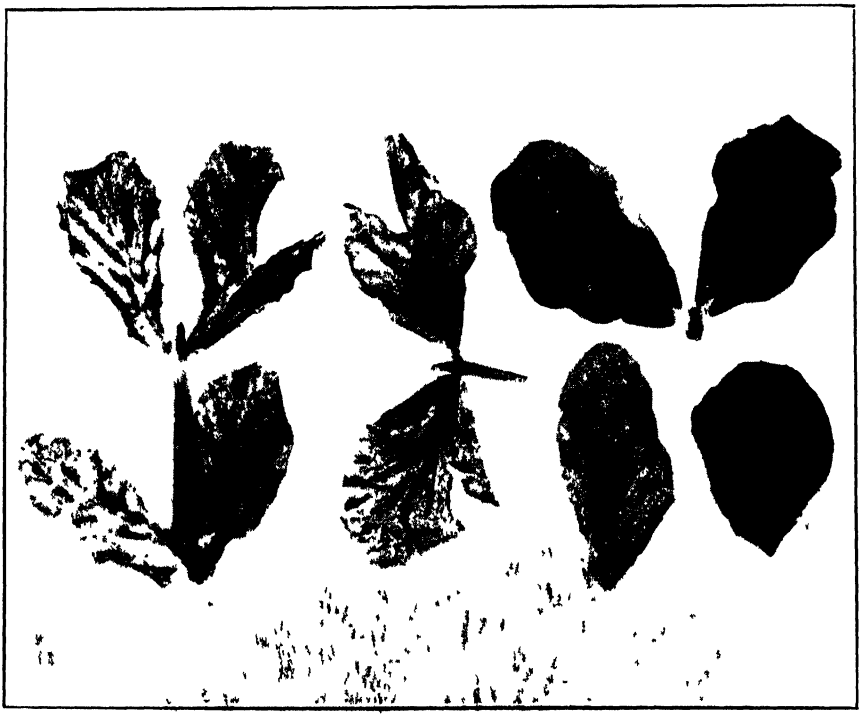

FIG. 4. Damage to leaflets that were approaching maximum size when the freezing occurred. Localized injury occurs in elongate, interveinal bands on the lower side, giving first the appearance of pale yellow enations and gradually becoming dark brown and somewhat glistening. The leaflets at the right are only slightly affected, while the ones at the left show extreme injury. In the center leaf the necrotic tissue has dropped out from some spots, leaving a ragged appearance. Specimens collected 6 weeks after the injury occurred.

noticeably disturbed. The freezing damage affects bands of interveinal parenchymatous cells of leaf or sepal. These bands appear first on the lower side of the leaflets as slightly erumpent tissue somewhat yellower than the normal color. With age they become gradually more conspicuous because of the change from light yellow eventually to deep brown, while the surface assumes a glossy appearance. At this advanced stage the lesions might readily be confused with those of bacterial blight (*Bacterium pisi* Sackett) were it not for their regular form and location in the leaf lamina and the lack of translucency. The necrotic tissue seldom extends to the upper surface, but when it does it may eventually drop out leaving a ragged appearance, as in the leaflets shown in the center of figure 4.

Stem cankers are commonly associated with frost injury. They occur particularly in the internode next below the injured leaf. The epidermal and cortical cells may be quite as sensitive as those of the last young leaf and certain of them are killed usually in a narrow band running parallel to the long axis of the internode. Since the remaining cells of this portion of the young stem are not affected, growth and expansion continues causing a rift in the superficial dead tissue, which has in the meantime

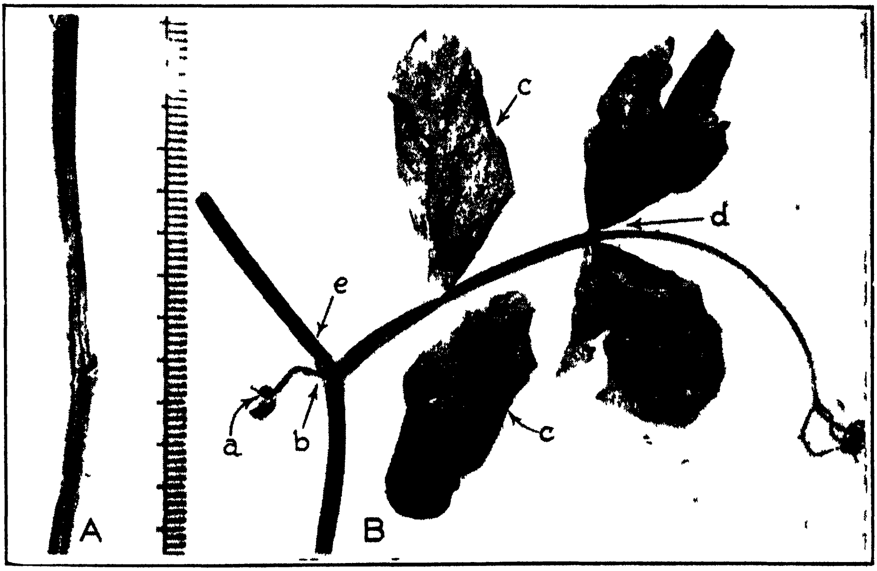

FIG. 5. Stem cankers caused by freezing injury. Specimens collected 6 weeks after the injury occurred. A. The original elongate band of dark superficial tissue has been rifted by the increase of stem diameter following growth of the underlying uninjured cells. B. The usual position of the stem canker is in the internode just below the injured leaf. In this instance the meristem and the last-formed very young leaf (a) were killed along with the young internode back to the next node (b). The sepals (c) were detached and moved back slightly when the photograph was made. They and the first pair of leaflets (d) were far enough advanced to respond in the manner illustrated in figure 4. The second pair of leaflets was suppressed entirely and the tendril somewhat malformed. The stem below the node was slightly injured and a canker formed. A bud at the base of the leaf escaped injury and expanded to form the new main stem (e).

become dark colored. A stem canker extending nearly the full length of two internodes is shown in figure 5, A.

The plant in figure 5, B, shows in one specimen many of the features of frost injury that have been discussed. It should be recalled that these symptoms are the ones that have accumulated over a period of six weeks following the injury. In this plant the growing point and the youngest leaf, which had just become differentiated, together with the young stem back to the next node were killed by the low temperature but remained attached during the following five weeks. A bud at this node escaped injury and its prompt growth continued, the original main stem forestalling stooling of the plant by growth of one or more buds near the soil line. The stipules at this node were only slightly affected, as shown by small dark bands. The leaf was injured moderately in that distinct frost bands developed in the first pair of leaflets, the buds of the second pair of leaflets were killed, and the tendril was retarded slightly. The stem tissue just below the node was injured and a characteristic canker formed at this point.

DISCUSSION AND SUMMARY

The damage to canning peas caused by late spring frosts is often more serious than that of which the grower is aware. This is due to the fact that plants are seldom killed and they recover rapidly from the effects of the injury. The delayed maturity of a portion of the plants seriously reduces the yield of peas at the prime canning stage, and the unevenness of growth is a limiting factor in satisfactory seed production. The responses of buds, leaves, sepals, and stems to freezing injury are of interest on the one hand because they illustrate the varied reactions of the growing organs to this sudden differential damage to the cells and, on the other hand, because they furnish diagnostic symptoms of this non-parasitic trouble that become very useful in detecting and estimating frost damage and in distinguishing it from other maladies.

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STATUS OF INVESTIGATIONS OF TOBACCO DOWNY MILDEW

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In 1921 a disease of cultivated tobacco known as downy mildew made its first appearance in Florida. Little was learned about the disease at that time, as the disease disappeared following the immediate employment of drastic methods of eradication. Ten years later, however, it reappeared in the same region, and during the first season of its reappearance, it became widely distributed. In the years that have since elapsed, downy mildew has encroached upon nearly all areas in the eastern United States devoted to the culture of tobacco. Although published reports of investigations of this disease are in accord in certain features, they are strikingly at variance in others, especially as regards the significance and application of the acquired knowledge to its control. Needless to say, much remains wholly unknown. It is the present purpose, therefore, to indicate from the experiences of the past 8 years the present status of our knowledge of essential features of the downy mildew of tobacco and of its causal organism.

ORIGIN OF THE PATHOGEN

There are no morphological features by which to distinguish between collections of the downy-mildew fungus, *Peronospora tabacina* Adam, whether from Australia or from the southeastern United States, when the sporangial and the oogonial stages of each are compared. The organism is definitely known to have been present in Australia as long ago as 1890, and doubtless it occurred there years earlier (1). From all available evidence there appears no reason to doubt that it is endemic to Australia. Some also entertain the opinion that it is native to North America. Reasons for this opinion are marshalled in the statements that follow. Foremost among them is the fact that Farlow (9), in 1885, collected a downy mildew on *Nicotiana glauca* near San Diego, California, which he identified as *Peronospora hyoscyami* de Bary, an organism first described in Europe (3) in 1863, on the related species *Hyoscyamus niger.* Our examination of Farlow's specimens shows that specific identification is uncertain because oospores are lacking. Apparently, the oospores of *P. hyoscyami* were first described in 1926, and have been shown to differ in size from those of *P. tabacina* (18). Moreover, Angell and Hill (1), and Wolf *et al.* (17) failed to secure infection of seedlings of *H. niger* grown among tobacco seedlings that became infected. Failure of *Hyoscyamus niger* to become infected by the tobacco pathogen leaves little likelihood that the organism collected by Farlow is *P. hyoscyami.*

The problem of identity of Farlow's collections and of endemism of *Peronospora tabacina* in North America is further complicated by the fact that Spegazzini (16), in 1891, described *P. nicotianae* on *Nicotiana longiflora* in Argentina. *P. tabacina* has been shown to be capable of infecting *N. longiflora* (1). Comparison of *P. nicotianae* from Argentina with *P. tabacina* from the southeastern United States shows that there are very obvious differences of specific rank, the former belonging in the section Calothecae and the latter, in Leiothecae (6, 18).

An additional complication arises from the fact that specimens of downy mildew on cultivated tobacco seedlings, collected in Texas in 1906 and on *Nicotiana bigelovii* in Nevada in 1914, have both been identified as *Peronospora hyoscyami.* Both collections consist of the sporangial stage of some species of *Peronospora* and neither can with certainty be specifically identified. There appears no reason for regarding them as *P. hyoscyami,* but it remains impossible to determine whether they are *P. nicotianae, P. tabacina,* or a third as yet undescribed species. Conclusive proof, therefore, of whether the tobacco-downy-mildew pathogen migrated from the western to the southeastern United States may never be forthcoming. The collection of oosporic material on *Nicotiana* in the West is highly desirable, and might furnish evidence that would contribute to a solution of the problem of endemism of *P. tabacina* in North America.

SOURCES OF INOCULUM

At first, the impression was widely current, not only among growers but even among investigators, that the initiation of tobacco downy mildew and the spread of the disease are and must remain shrouded in mystery. It has been well established, however, from studies on the life history of the pathogen that it possesses 2 stages, an oosporic stage, by means of which the organism survives from one season to the next, and a sporangial stage, by means of which it is disseminated. Oospores constitute the source of inoculum for primary infections, and sporangia that for secondary infections (7, 18). Primary infections are further characterized (a) by their occurrence on sites occupied the preceding year by diseased seed beds; (b) by the appearance of the disease (production of sporangia) 9 to 17 days prior to sporulation in any near-by bed on a new site; and (c) by the occurrence in old-bed sites of a single infected seedling or, at most, a few groups of 3 to 4 diseased seedlings. Primary infections *do not occur* in all seed beds sown on old sites. Secondary infections are first apparent within 9 to 17 days after sporulation on primarily infected seedlings. The interval may be longer toward the southern part of the range. Secondary infections are first apparent in any locality in primarily infected beds on seedlings that did not exhibit primary infection. Subsequently, secondary infection may appear at approximately the same date either in new beds located near primarily infected old ones or in old beds that escaped primary infection.

The earliest outbreaks of tobacco downy mildew in every locality, once the disease has appeared in that area, have *without exception* taken place in beds on old sites. Some maintain that outbreaks occur as early in beds on new sites as in those on old sites. Those who hold to this opinion have failed to notice primary infections and have seen only secondary ones. That primary infections may occur in old bed sites is substantiated by our observations for 7 seasons, by the occurrence of oospores in the life cycle of the tobacco pathogen, and by analogy with the known function of oospores in the cycle of development of other downy mildews.

DISSEMINATION OF INOCULUM

Species of *Peronospora* are adapted to a terrestrial habitat, for, unlike closely related genera, they lack motile zoospores and their sporangia function as conidia. For these reasons, as might be anticipated, the dissemination of the tobacco pathogen is accomplished mainly by wind-borne sporangia. Man is a vector of secondary importance. It has been demonstrated by means of spore traps (7, 17) that sporangia may be transported by currents of air for distances of several miles. It seems reasonable, therefore, to assume that air-borne sporangia served to spread the fungus throughout Florida and Georgia in 1931, and thence, in turn, into South Carolina, North Carolina, and Virginia. Thereafter, the pathogen was more widely distributed within each of these States and extended its range into Tennessee, afterwards into Kentucky, and then into southern Ohio and Indiana. In 1938 it probably was disseminated by air currents from the region north of the Ohio River into Ontario, Canada. In another direction, meanwhile, it spread northward from Virginia into Maryland and Pennsylvania, and then, in 1937, from the latter State into Connecticut and Massachusetts. Indirect evidence of air-borne inoculum is provided both by the pattern and rate of its spread during the development of an epiphytotic in any locality. Other indirect evidence comes from analogy with other species of downy mildew, as for example, *Peronospora destructor* (Berk.) Caspary on onion (13). Many sporangia of this organism, entrapped to a height of 1500 feet over diseased onion fields, were found capable of germination.

SPORANGIA

Two observations have been made regarding the sporangia of the tobacco pathogen whose significance is quite generally unappreciated. First, they are produced in countless numbers during any favorable period of a few days duration, and second, the sporangial cycle is repeated in from 4 to 7 days. In consequence, a primarily infected bed can, within 3 weeks, provide sufficient inoculum to initiate an outbreak involving all seed beds in an area comprising several hundred square miles.

Sporangial formation occurs at daybreak (17), but the proximate stimuli that cause their production at this time of day remain unknown.

OOSPORES

Certain species of downy mildews are not known to possess an oosporic stage. In the case of the tobacco pathogen, however, oospores have been noted in necrotic leaves during each of 7 seasons. Since they have been collected in Connecticut, Maryland, North Carolina, South Carolina, and Virginia, they may be presumed to occur wherever the pathogen exists. Essentially nothing, however, has been learned regarding the influence of temperature, moisture, and age of lesions on oospore formation, nor of the length of time required for oospores to develop. Aside from the existence in dissected materials of oogonia to which antheridia were applied, little is known about the origin of oospores. The cytological phenomena attendant upon fertilization, nuclear fusion, and reductional division are wholly unknown. Germination of oospores has been observed only a few times in hundreds of trials (18). At present the oospores must be regarded as very refractory to germination. Once a technique has been developed whereby they can be made to germinate at will, penetration of leaf tissues by the germ tubes and other stages of infection can then be observed. To date, these things have not been accomplished.

RELATION OF WEATHER TO THE DISEASE

Early observations that have subsequently been substantiated indicate that the destructiveness of tobacco downy mildew is conditioned by temperature and moisture conditions (8). The erratic seasonal behavior of this disease in date of the first outbreak, rate of spread, amount of damage occasioned, rate of recovery of infected seedlings, reinfection of seedlings in the beds or of plants in the field are correlated with weather conditions. Evidence is lacking to show that the pathogen varies in aggressiveness. Variation in the fungus, therefore, can not be used to account for differences in destructiveness from year to year, and the disease does not appear to be more benign than formerly. There is no reason to believe that the fungus will again disappear, for it has recurred during each of 8 years. It has not relinquished any invaded territory during this period. The amount of loss that it may cause is wholly unpredictable, since it is governed by weather.

Experimentation on relation of weather to tobacco downy mildew has been of two kinds, one performed by use of apparatus for making and controlling temperature and moisture conditions (2, 4, 10), and the other by measuring conditions occurring in seed beds (8). Neither kind measured the conditions existing immediately at the leaf surface or inside the leaves, where the pathogen functions; hence, neither delimits precisely the effective range in limits of temperature and humidity factors. If the data from both kinds of experimentation are interpreted with this in mind they still remain valuable in understanding the course of the disease.

RESISTANCE TO THE ORGANISM

In some seasons seedlings that have survived an attack may not again become diseased, a fact that has been interpreted as showing that a degree of immunity is acquired as a result of the disease. In other seasons the seedlings, while still in the seed bed, may have one or more recurrences of the disease, or it may recur soon after they have been transplanted. Recurrence of downy mildew appears to be causally correlated with weather factors, but other as yet unknown conditions may be primary causes. It is indicated that one might profitably employ this tobacco disease in researches on immunity in plants.

CONTROL OF THE DISEASE

To all who have devoted themselves to investigations of tobacco downy mildew, it is quite apparent that its control constitutes a perennial problem. Three general types of control measures have, to date, been given consideration. They are: (a) planting a seed bed-areas in excess of normal requirements in the hope that a sufficient number of seedlings will survive to permit the planting of the crop; (b) spraying with fungicides (5, 10), of which copper oxide-oil affords a degree of protection; and (c) fumigation with volatile compounds, of which benzol has been given most consideration (11, 12, 14, 15, 19).

Experience has shown that in most seasons enough seedlings survive attack in the beds to permit the planting of the desired acreage, when 2 or more times the normal seed-bed area is sown. This practice, however, does not always provide sufficient seedlings in all localities.

The nature of the disease is such that one would not expect to secure satisfactory control by means of dusts or sprays. Some degree of control, however, has been secured when the weather is unfavorable for a serious outbreak. The results of experiments in areas where spraying has been attempted do not indicate that sprays can be relied upon to give adequate protection.

The employment of gaseous fungicides to control tobacco downy mildew is indicated by the nature of the disease. Benzol has been successfully used for this purpose, first in Australia, then in the United States. Excellent control also has followed the use of other related volatile substances, such as monochlorobenzene and paradichlorobenzene. Further experimentation should involve these and other related compounds.

Recent studies with benzol involve the volume-percentage vapor concentrations necessary for fungistatic and fungicidal action, and the influence of the various modifying factors upon vapor concentration (14, 19). Since benzol vapor acts by virtue of its being dissolved in water, it becomes highly desirable comprehensively to investigate such matters as efficient and economical methods of applying benzol, times of application, and minimal duration of the periods of exposure for fungistatic and fungicidal action.

SUMMARY

Consideration is given to the status of our knowledge regarding endemism of *Personospora tabacina,* sources of inoculum, dissemination of sporangia, and the relation of weather to the disease. The lack of essential information regarding the oospore stage and regarding possible resistance of recovered seedlings is indicated. Although the use of benzol provides a means of control of tobacco downy mildew, fundamental problems relative to its use remain unsolved.

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A SIMPLE METHOD OF MEASURING THE INTERFACIAL FRICTION OF DUSTED SEEDS[1]

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INTRODUCTION

The use of chemical dusts for the protection of seeds against decay and seedling diseases is an established practice. The addition of the chemical dusts to the seed, however, has usually altered the interfacial friction of the seed. No adequate method of measuring this friction has been devised. Published results deal with the rate of movement of dusted seeds through the planter—not with the friction developed. Arnold and Horsfall,[2] Koehler and Shawl,[3] Leukel,[4] and Pearson,[5] have studied the effect of various chemical dusts on the flow of seeds through various types of planting machinery. The rate of flow varies, of course, with the friction, but differences observed are clearly not of the same order of magnitude as those that can be felt when the planter is operated by hand. Present methods have other objections, as well. (a) A large amount of seed is required; (b) considerable equipment and space are necessary; (c) time required to make one determination is too long. Frequently, the question arises as to whether one chemical dust will increase the interfacial friction of dusted seed more than another. If so, how much difference is present? Is one grade of graphite more efficient in reducing interfacial friction than another? Do other suggested lubricants act as efficiently as graphite? What is the effect on interfacial friction of materials used as fillers in different chemical dusts?

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[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 286, August 2, 1938. Part of a Research Project conducted in cooperation with the Crop Protection Institute.

[2] Arnold, Earl L. and James G. Horsfall. Use of graphite to prevent clogging of drills when sowing dusted pea seed. N. Y. State Agr. Exp. Stat. Bull. 660. 1936.

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[4] Leukel, R. W. Relation of dust fungicides to flow of small grains through drills and to drill injury. U. S. Dept. Agr. Circ. 119. 1930.

[5] Pearson, O. H. The effect of various dusts upon the rate of seeding of various vegetable seeds. Proc. Am. Soc. Hort. Sci. 34: 559–561. 1937.

To answer these questions easily, rapidly, and accurately, a new method of measuring the interfacial friction of dusted seed has been developed. This method requires only a small amount of such seed. The necessary equipment usually is found in any laboratory.

THE METHOD

The equipment includes a 300 cc. tall beaker, a plunger of one-half inch wooden dowelling about 12 to 15 inches long with a suitable marker attached 3 inches from one end (a beaker brush with a plain wooden handle ½ in. diameter is satisfactory), and a spring type platform household scales that reads up to 24 pounds. Sufficient dusted or nondusted seed is added to fill the beaker to the bottom of the lip. This gives a column of seed 2½ in. in diameter and 4 in. deep. For peas and wheat, 250 g. are required, while for beets only 31 g. are necessary. The beaker of seeds is placed on the scales and the indicator needle adjusted to zero. The plunger is pushed into the seed at a constant rate to a depth of 3 in., and the greatest deflection of the indicator needle is recorded by an assistant. The figure recorded is the pounds' pressure required to thrust the plunger into the seed to a depth of 3 in. The sample of seed is loosened and rearranged between each trial by inverting the beaker in the hand. When the method was first used, each test comprised 50 trials. However, an analysis of the number of trials required for the least probable error indicated that 30 trials were as satisfactory as 50. Consequently, in subsequent tests, only 30 trials were used to give an average measurement of the interfacial friction. A statistical analysis has indicated that a difference of 1 lb. between any 2 tests is significant.

RESULTS

The data accumulated over a period of nine months by using the above method are presented in table 1.

With regard to peas, non-dusted large wrinkled seed (Wilt Resistant Perfection) showed more interfacial friction than non-dusted small wrinkled seed (Wisconsin Early Sweet). The interfacial friction of a non-dusted small wrinkled seed was about the same as that of small smooth seed (Alaska). The addition at equal dosages of red copper oxide, 2% ethyl mercury chloride and ethyl mercury phosphate to pea seed resulted in a marked increase in the interfacial friction. When ethyl mercury phosphate was used on Wilt Resistant Perfection seed, an increase in dosage resulted

TABLE 1.—*Interfacial friction of dusted seeds*

Material	Lubricant[a]	Pea seed friction[b]						Wheat, var. Forward		Beet seed, var. Detroit Dark Red	
		Dosage, per cent by weight	Wisconsin Early Sweet		Wilt Resistant Perfection. Moisture content, per cent		Alaska	Dosage, per cent by weight	Friction[b]	Dosage, per cent by weight	Friction[b]
			8/5/37	9/19/37	12	8.8					
Check	....	....	2.92	2.98	6.81	5.93	3.12	....	1.31	....	9.49
Red copper oxide	....	0.25	7.83	8.79	22.10	11.60	6.57	0.21	4.80	2.0	11.10
do	Graphite No. 1, flake	0.25	3.58	3.80	5.79	....	3.69	0.21	2.23	2.0	10.20
do	Graphite No. 2, amorphous	do	....	....	8.15	....	....	....	....	....	....
do	Graphite No. 3, flake	do	....	....	6.85	5.51	....	....	....	....	....
do	Graphite No. 4, amorphous	do	....	....	7.56	....	....	....	....	....	....
do	Graphite No. 5, amorphous	do	....	....	9.22	6.78	....	....	....	....	....
do	Whiting	do	....	....	....	....	6.83	....	....	....	....
2% Ceresan	....	0.25	7.53	....	16.00	12.00	6.22	....	....	....	....
do	Graphite No. 1, flake	0.25	....	....	....	....	4.70	....	....	....	....
New Improved Ceresan	....	0.05	4.83	....	8.19	....	....	0.042	1.90	....	....
do	....	0.10	....	....	10.40	....	....	....	....	....	....
do	....	0.25	....	....	11.50	....	....	....	....	....	....
do	....	0.40	....	....	13.10	....	....	....	....	....	....
Copper carbonate	....	....	....	....	....	....	....	0.21	6.81	....	....
Semesan	....	....	....	....	....	....	....	....	....	2.0	12.40
Zinc oxide	....	....	....	....	....	....	....	....	....	2.0	16.30
Talc	....	0.25	....	....	....	....	5.78	0.21	2.47	2.0	14.00

[a] Dosage of lubricant is one-half that of the chemical dust.

[b] Pounds pressure required to push plunger 3 inches into seed. Average of at least 30 trials.

in an increased interfacial friction. Flake graphite (table 1, No. 1 and 3) of 325-mesh size was more efficient in reducing the interfacial friction of dusted seed than amorphous graphite (table 1, No. 2, 4 and 5) of equal particle size. Whiting, which was proposed by a manufacturer as a lubricant, did not reduce the interfacial friction of dusted seed. An increase in the moisture content of pea seed increased the interfacial friction of dusted and nondusted seed. It also increased interfacial friction of dusted seed to a greater degree when a lubricant was not used than when it was (Wilt Resistant Perfection). These results are in agreement with those reported by other workers.[6]

In the case of wheat, the addition of copper carbonate showed a greater increase in interfacial friction than the addition of red copper oxide at equal dosage. The addition of graphite to the red copper oxide-dusted wheat reduced the interfacial friction materially. Arnold and Horsfall[7] have obtained similar results, while Leukel[8] also has shown that copper carbonate increases the interfacial friction of wheat seed. Friction was increased about 30 per cent, even with the low dosage of ethyl mercury phosphate recommended for wheat.

Beet seed has shown an increase in the interfacial friction from the addition of such dusts as red copper oxide, Semesan and zinc oxide. The addition of graphite to the red copper oxide-dusted seed gave a slight reduction in the interfacial friction. Pearson[9] has indicated that red copper oxide-dusted beet seed showed a slight acceleration in the seeding rate, while that dusted with zinc oxide showed a decrease in the seeding rate.

DISCUSSION

This method of measuring the interfacial friction gave results in essential agreement with those published. It is probable that the results obtained with it will indicate what will happen when such seed is used in planting machinery. Occasionally, red copper oxide-dusted pea seed have given readings beyond the limit of the scales. It is these odd cases, when the seeds become interlocked and require considerable pressure to force them past each other, that cause the breaking of the internal force-feed drill cups as reported by Arnold and Horsfall.[10] These occasional cases of extraordinary friction are not measurable by rate-of-seeding tests. Thus, when any seed is tested by this method and shows an occasional high reading, trouble may be expected to develop in the drill. If, however, the interfacial friction is higher than normal and no large variations are observed, it can be expected that the seeding rate will be reduced without undue trouble with the machinery. As indicated in table 1, certain materials used as fillers in the different chemical dusts, such as talc, will, themselves, be responsible for an increase in the interfacial friction of the dusted seed.

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[6] See footnotes 2 and 5.

[7] See footnote 2.

[8] See footnote 4.

[9] See footnote 5.

[10] See footnote 2.

SUMMARY

A simple method of measuring the interfacial friction of dusted seeds is described. A plunger is thrust into a beaker full of seeds sitting on a spring platform scales. The interfacial friction is measured in pounds by the indicator needle on the scales.

Results are given that indicate the reliability of this method, in that the results are in agreement with what is observed in the field and with results reported by other workers.

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PATHOGENIC RACES OF ACTINOMYCES SCABIES IN RELATION TO SCAB RESISTANCE[1]

J. G. LEACH, PHARES DECKER, AND HANNAH BECKER

(Accepted for publication Sept. 12, 1938)

Common scab of potatoes, caused by *Actinomyces scabies* (Thax.) Güssow, is probably the most important limiting factor in the production of high-quality potatoes, and it also is one of the most difficult potato diseases to control. For this reason the development of scab-resistant varieties is one of the primary goals in most potato-breeding programs. In recent years several varieties or selections have been reported as having a relatively high degree of resistance to scab, and many of them are being used in breeding with the view of combining scab resistance with other desirable characters.

Stevenson and Clark (3) in 1934 described a seedling, U.S.D.A. 44537, which was resistant in comparative tests in Maine, but, when tested in Minnesota, was relatively susceptible (1). Similarly, the Minnesota seedling 5–10–1, reported as resistant in a 5-year comparative test in Minnesota (1), was susceptible when grown in certain other localities. This variation in resistance was noted by Darling (1), who suggested that "It is possible that this may be due to different physiologic forms of *Actinomyces scabies.*" Evidence proving the correctness of this suggestion has been obtained and is reported here.

In 1935, in a trial plot at Duluth, Minnesota, several tubers of the seedling 5–10–1 were found affected with a few lesions of scab of a severe pitted type. Infection of this severe type had not been observed previously on this selection, which had been tested for 5 years on heavily infested peat soil at Coon Creek, near St. Paul, Minnesota. The pathogen was isolated from the severe lesions and from similar lesions on Irish Cobbler tubers grown on the peat-soil at Coon Creek. The cultures of the two isolates were similar

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[1] Paper No. 1640 of the Scientific Journal Series, Minnesota Agricultural Experiment Station.

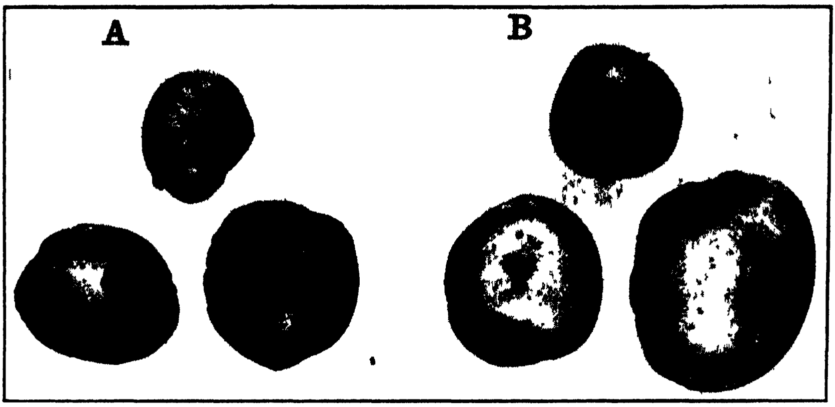

FIG. 1. Representative tubers of seedling 5–10–1 grown in soil infested with two different races of the scab pathogen. A. Race No. 1. B. Race No. 2.

but not identical when grown on several different media, but, when used for inoculating the selection 5–10–1, they were found to differ strikingly in pathogenicity, as shown in figure 1. The inoculations were made in the greenhouse in the winter of 1935–36.

In order to compare further the differences in pathogenicity of these isolates, they were tested again in 1936–37 on the following differential hosts: (a) Warba, a very susceptible variety; (b) Jubel, a German scab-resistant variety; (c) Minnesota seedling 5–10–1; (d) U.S.D.A. seedling 44537; (e) Ackersegen, a German scab-resistant variety, and (g) Arnica, a German scab-resistant variety. The tests were made in the greenhouse in 12-inch pots of soil that had been steam-sterilized and tested for freedom from *Actinomyces*. The inoculum was grown in quart fruit jars on a mixture of loam and peat. One quart of "inoculated" soil was added to each 12-inch pot immediately before the tubers were planted. The seed tubers were disinfected in acid mercuric chloride solution just before planting. Each isolate and each differential host was used in duplicate with a non-inoculated check, and the pots were randomized as to location on the greenhouse bench. The results of the test are illustrated in figure 2. It will be noted that Warba is very susceptible to both isolates, 100 per cent of the surface of the tubers being scabbed. U S D A. seedling 44537 is moderately susceptible to both strains. Arnica is slightly susceptible and reacts essentially the same to both isolates. Seedling 5–10–1, however, is highly resistant to isolate 2 and very susceptible to isolate 1. Jubel also is a differential host, being moderately susceptible to isolate 1 but highly resistant to isolate 2.

This experiment was repeated in 1937–38, except that only 4 of the differential hosts were used, namely, Warba, Jubel, 5–10–1, and Ackersegen. The results of the second test were the same as those obtained previously and representative tubers are illustrated in figure 3.

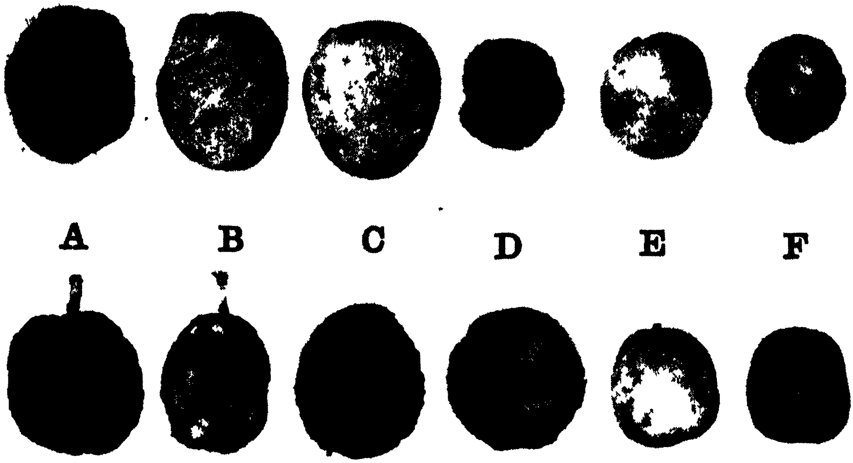

FIG. 2. The reaction of 6 selections of potatoes to two races of the scab pathogen (1936–37). Upper row, Race No. 2. Lower row, race No. 1. A. Warba. B. Jubel. C. 5–10–1. D. U. S. 44537. E. Ackersegen. F. Arnica.

These experiments, although not extensive, demonstrate conclusively that the two isolates are different pathogenic races of the scab pathogen. They also show that the difference in scab resistance observed in the seedling 5–10–1 in different localities is caused by the presence of different

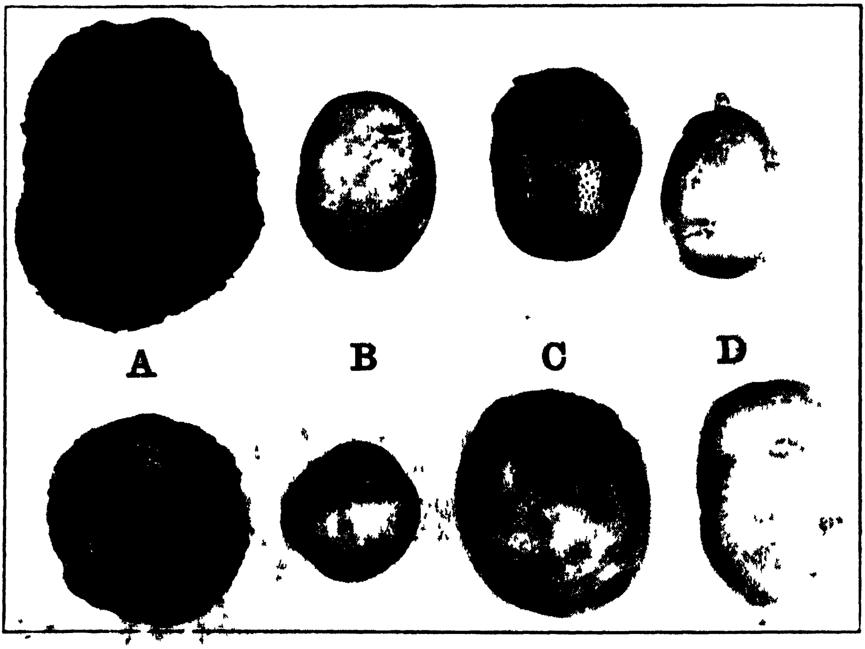

FIG. 3. The reaction of 4 selections of potatoes to two races of the scab pathogen (1937–38). Upper row, Race No. 2. Lower row, Race No. 1. A. Warba. B. Jubel. C. 5–10–1. D. Ackersegen.

pathogenic races. The susceptibility of U.S.D.A. seedling 44537 in Minnesota and its resistance in Maine probably can be explained on the same basis, although this seedling was equally susceptible to both of the isolates studied. The significance of these facts in the problem of breeding for scab resistance is obvious. It will be necessary to test breeding stock and potential new varieties to many different races of the scab pathogen Whether this can be accomplished most practically through the inoculation of a single plot of ground with many isolates of the pathogen, or by cooperative tests on many plots of soil in various sections of the country remains to be determined. Both methods undoubtedly will be found useful. The program of breeding for disease resistance will be furthered by recognition of these facts.

Previous workers, Waksman (4), Wollenweber (6), Millard and Burr (2), Wingerberg (5), have recognized numerous strains of *Actinomyces,* including those causing potato scab, but the strains have been distinguished largely on cultural characteristics. Millard and Burr described 11 isolates of *Actinomyces,* differing in cultural characters and type of lesions produced, which they interpreted as 11 distinct species. They described several different types of scab lesions and concluded that the "type of scab is dependent on the infecting *Actinomyces* species. The variety of potato may modify but can not materially change this type." On the basis of results presented here and on several years' work with large numbers of potato seedlings, the latter statement appears not to be fully justified. The scab lesion is the result of a reaction between the pathogen and the host tissue and may be influenced as much by the host as by the pathogen. Furthermore, isolates of *Actinomyces* that are similar in cultural characters may be distinguished on the basis of pathogenicity or the type of lesions caused on the same variety, and are, therefore, properly designated as pathogenic races.

The results presented here also have some significance in the study of the nature of scab resistance. Darling (1) made a comparative study of the resistance of 5–10–1 and a susceptible seedling, as observed on the peat soil at Coon Creek (race 2), and concluded that, "Insofar as the study of the nature of scab resistance has been carried the evidence points towards the structure of the lenticel as a determining character although other factors may be involved. . . . The lenticels of the resistant seedling studied were much smaller than those of the susceptible seedling. Their cells were also smaller, more compact, and oblong."

The data presented here indicate that physiologic factors also are involved in resistance. The different reaction of the test varieties to the two races can be explained satisfactorily only in this way. However, it has been observed that the selection 5–10–1 usually has a much smaller number of lesions than other varieties under comparable conditions, regardless of the races of scab present. This is believed attributable to the morphological resistance afforded by the smaller, more compact lenticels. This type of resistance can be overcome to some extent by a very heavy artificial inoculation of the soil, even with race 2 to which it is also physiologically resistant. This was demonstrated in an extensive experiment in the summer of 1936, when the plants were grown in extremely heavily inoculated soil of two types in 12-inch pots set in the soil out of doors. The soil in the pots was kept relatively dry, and very heavy infection was obtained on 5–10–1, as shown in figure 4. It will be noted that, although there are many lesions on the tubers grown in soil heavily inoculated with race 2, almost all of the lesions are of the shallow, resistant type. This experiment also confirmed the pathogenic difference of the two isolates, but it was not entirely conclusive in regard to other aspects because considerable scab developed in the checks grown in noninoculated, steam-sterilized soil. This contamination probably resulted from wind-blown soil or perhaps incomplete sterilization, but it invalidated many of the conclusions that might have been drawn from the experiment.

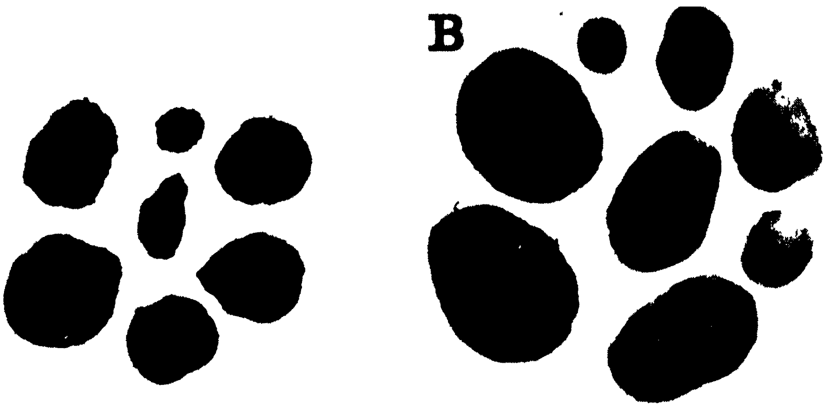

FIG. 4. The reaction of selection 5–10–1 to very heavy inoculation with two races of the scab pathogen. A. Race No. 1. B. Race No. 2. Note the different types of lesions.

SUMMARY

The occurrence of two pathogenic races of *Actinomyces scabies* in reported. The susceptibility of potato seedlings, previously reported as resistant, is explained on the basis of different pathogenic races of the pathogen. The significance of the recognition of pathogenic races in the problem of breeding for resistance and in the study of nature of resistance is discussed.

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3. STEVENSON, F. J., and C. F. CLARK. New potato varieties. Amer. Pot. Jour. 11: 85–92. 1934.
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5. WINGERBERG, FRITZ. Studien über den gewöhnlichen Kartoffelschorf und seine Erreger. Kühn-Arch. 33: 259–295. 1932.
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CHEVRON, A BARLEY VARIETY RESISTANT TO STEM RUST AND OTHER DISEASES

R. G. SHANDS

(Accepted for publication Sept. 12, 1938)

Chevron, C.I. 1111,[1] is a variety of *Hordeum vulgare pallidum.*[2] Seed was obtained from the U. S. Department of Agriculture, and observations have been made on this variety for 5 years at Madison, Wisconsin. It is of the spring growth habit, has resistance to several diseases, and has other characters of value to the plant breeder.

The barley accession records[3] of the U. S. Department of Agriculture show that a lot of barley seed, C.I. No. 1111, S.P.I. No. 38061, was presented to the U. S. Department of Agriculture in 1914 by Dr. Albert Volkart of the Swiss Seed Experiment Station at Zurich, Switzerland, through the U. S. Consul. This barley was an unimproved domestic variety obtained from Vorrenwald Eich, in Canton Lucerne, Switzerland, and was described as a "Four-lined spring barley." It was sown in bulk at Arlington Farm, Virginia, in the autumn of 1914. In 1915, the seed was divided, one part being sent to St. Paul, Minnesota, and the other to Chico, California. A selection from the part sent to Chico was named Chevron in 1918 and retained the number C.I. 1111. Peatland, C.I. 5267, originated as a selection made in 1916 at the Minnesota Agricultural Experiment Station from the other part of the seed.

The variety Chevron was among a small number sown late in 1935 at Madison, Wisconsin, and at that time was first observed to possess resistance to stem rust (*Puccinia graminis* Pers.). Susceptible varieties had infections of 20 to 25 per cent, whereas Chevron and Peatland were rust-free. In 1937 a severe natural epidemic of stem rust occurred in the barley nursery at Madison, Wisconsin. An average severity of 74.8 per cent infection was obtained for 75 varieties grown in a plot of rod rows. Chevron was among these varieties and severity of infection ranged from a trace to about 2 per cent with an average of 1 per cent. Uredia were very small and only a small number succeeded in breaking through the epidermis. The degree of resistance in Chevron appeared to be approximately the same as that in Peatland.

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[1] Accession number of the Division of Cereal Crops and Diseases, United States Department of Agriculture.

[2] Harlan, Harry V. The identification of varieties of barley. U. S. Dept. Agr. Bull. 622. (Professional paper) 1918.

[3] Information on the history of Chevron was supplied by Dr. G. A. Wiebe of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Progenies of 25 Chevron plants taken at random were grown in 10-foot rows. All of the progenies were uniformly resistant to stem rust and appeared similar in growth habits. Chevron was observed in the Agricultural Experiment Station plots at Ames, Iowa, and at Waseca, Minnesota, where severe epidemics of stem rust also occurred in 1937. At each of these places this variety was resistant to stem rust.

Powers and Hines[4] reported that the stem-rust resistance of Peatland was inherited as a single dominant factor. Observations at Madison, Wisconsin, in 1937, with back crosses involving Chevron, indicated that rust resistance of this variety is inherited in a similar manner. In back crosses, where the susceptible recurring parents were Wisconsin Barbless (Pedigree 38), Velvet, and 2 smooth-awned hybrid selections, a total of 61 progenies from F_1 plants were observed for stem rust reaction. The results are given in table 1. The values of P for the individual back crosses were satisfactory. The combined results from the 4 back crosses gave a total of 32 heterozygous and 29 homozygous susceptible progenies. A total X^2 value of 0.1475 with a P value between 0.80 and 0.70 was obtained for a theoretical 1:1 ratio. In 3 crosses where Wisconsin Barbless (Pedigree 38), Velvet, and a smooth-awned hybrid selection were the female parents and Chevron the male parent, stem rust data were obtained on a total of 38 F_3 smooth-awned families. These families segregated as follows: 9 homozygous resistant, 23 heterozygous, and 6 homozygous susceptible. The X^2 test for goodness of fit of the total to a theoretical ratio of 1:2:1 gave a value of 2.1579 with a P value between 0.50 and 0.30. Further data were obtained in 1938 from a cross between Oderbrucker (Pedigree 5–1) and Chevron. The F_2 population of 356 plants segregated into 275 resistant and 81 susceptible plants. This segregation for stem rust fitted the 3:1 ratio for a single factor, as judged by a X^2 of 0.9588 with a P value between 0.50 and 0.30.

TABLE 1.—X^2 *for goodness of fit to a theoretical 1:1 ratio for stem rust resistance of progenies from* F_1 *plants of back crosses*

Back cross	Heterozygous	Homozygous susceptible	X^2	P lies between
H23 (Wis. Ped. 38 x Chevron) x Wis. Ped. 38	12	14	.1538	0.70 and 0.50
H29 (Velvet x Chevron) x Velvet	12	6	2.0000	.20 and .10
H35 (X152 x Chevron) x X152	5	8	.6922	.50 and .30
H37 (X169 x Chevron) x X169	3	1	1.0000	.50 and .30
Total	32	29	.1475	.80 and .70

Chevron has been tested, by the writer, along with other varieties, for its reaction to the scab fungus, *Gibberella saubinetii* (Mont.) Sacc. In experiments conducted over a period of several years it has been consistent in its relatively high resistance to scab. The variety has a stiff straw about equal in stiffness to that of Peatland. Its heading date is medium late, approximating that of Wisconsin Barbless (Pedigree 38). The size of kernel in normal years is somewhat smaller than those of the common commercially grown varieties. The hull sticks tightly to the kernel. Results, although not conclusive, indicate that this variety will not be a good yielder. Under heavy epidemic conditions of certain diseases, Chevron may yield more and have better quality than commercial varieties susceptible to these diseases. However, in results obtained at the New York Experiment Station, at Ithaca,[5] its average yield in rod-row tests fell in the top third of barley varieties.

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[4] Powers, LeRoy and Lee Hines. Inheritance of reaction to stem rust and barbing of awns in barley crosses. Jour. Agr. Res. [U. S.] 46: 1121–1129. 1933.

Mains and Martini[6] reported Chevron susceptible to leaf rust and highly resistant to 3 physiologic races of mildew. Tidd[7] reported that it was highly resistant to powdery mildew, physiologic races 6 and 7. Under field conditions at Madison, Wisconsin, Chevron has been susceptible to leaf rust, but it has been resistant to natural infections of mildew in the field, as well as in the greenhouse.

In work not yet published, H. L. Shands has found Chevron moderately resistant to the forms of the stripe fungus, *Helminthosporium gramineum* Rabh., that he used in tests at Madison, Wisconsin. His experiments indicate that this variety is susceptible to the sporidium-forming smuts of barley. The reaction of Chevron to the floral-infecting loose smut has not yet been determined.

The known sources of resistance to stem rust in barley are very limited. In a program [illegible] barley improvement, it is advantageous for the plant breeder to have several sources of material for each of the desired characters. The program may be materially hastened and made less difficult if several combinations of characters within each of the linkage groups are available. Since Chevron is resistant to several diseases and has other desirable qualities, it may be a useful parent in producing a barley adapted to conditions in the upper Mississippi valley region of the United States.

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U. S. DEPARTMENT OF AGRICULTURE,
IN COOPERATION WITH THE UNIVERSITY OF WISCONSIN,
MADISON, WISCONSIN.

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[5] Harlan, Harry V., P. Russell Cowan, and Lucille Reinbach. Yields of barley in the United States and Canada, 1927–31. U. S. Dept. of Agr. Tech. Bull. 446. 1935.

[6] Mains, E. B. and Mary L. Martini. Susceptibility of barley to leaf rust (*Puccinia anomala*) and to powdery mildew (*Erysiphe graminis hordei*). U. S. Dept. Agr. Tech. Bull. 295. 1932.

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PHYTOPATHOLOGICAL NOTES

A Spore Isolator Combining Some of the Advantages of the La Rue and Keitt Methods.—For some time the writer has been using a device for obtaining single spore isolates that combines the advantages of the La Rue[1] spore isolator and the Keitt[2] "biscuit cutter." As shown in figure 1, A, the device is essentially a "biscuit cutter" mounted vertically in a threaded brass plug, screwed on the microscope in place of the oil-immersion objective. In practice it has been found advisable to make the "cutter" about 3/4 mm. in diameter or slightly less than the microscope field under the low power. The cutter can be centered in a few minutes by bending the wire so that it will mark off a circle concentric with the low-power field of the microscope. Once it is centered, it will remain so almost indefinitely. The procedure used to obtain single-spore cultures with this device is as follows:

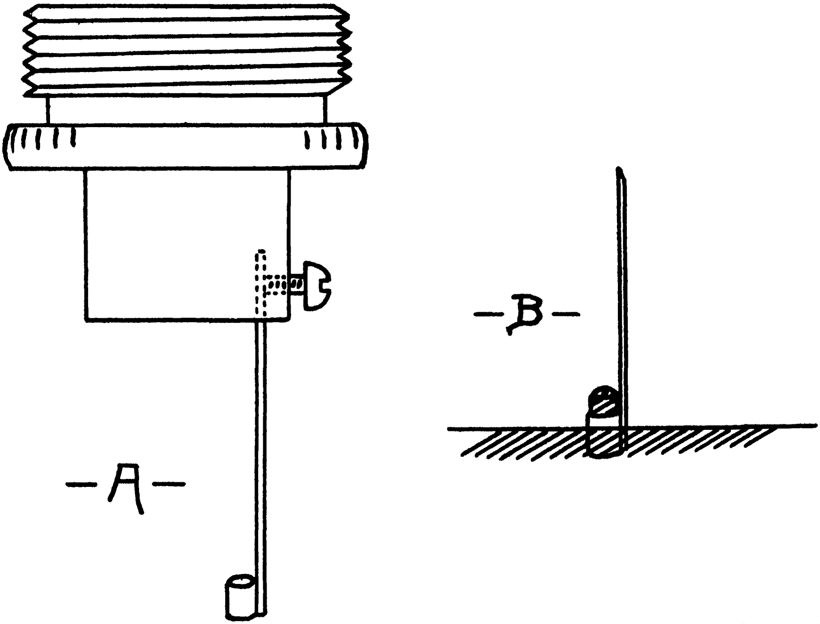

FIG. 1. A. Diagrammatic sketch of the spore isolator comprising a brass plug, which takes the place of a microscope objective. To this is attached a platinum wire with a "biscuit cutter" on the lower end. B. Pushing the disc of agar through the top of the cutter.

1. Spore dilutions in sterile water are streaked with a sterile wire loop across the surface of agar in a Petri dish.

2. After the water has dried sufficiently for the spores to remain in place, a spore, sufficiently separated from other spores, is located.

3. The revolving nose piece of the microscope is then turned so that the cutter (previously flamed) is swung into position over the spore. It is then

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[1] La Rue, C. D. Isolating single spores. Bot. Gaz. 70: 319–320. 1920.

[2] Keitt, G. W. Simple technique for isolating single-spore strains of certain types of fungi. Phytopath. 5: 266–269. 1915.

moved downward until a circle is marked off in the agar around the spore, when it is raised and swung to one side.

4. The position of a spore to be isolated now is noted, as well as that of any nearby spores (all in the same plane on the surface of the agar).

5. When the operator is certain that only one spore lies within the circle and that there are no other spores too close for safety, the cutter is again swung into place and this time lowered sufficiently so that a column of agar fills the cutter, forming a tiny disc with the single spore resting on its surface.

6. This agar disc within the cutter usually breaks off from the agar below when the cutter is raised. The breaking off of the agar disc can be assured by a slight horizontal movement of the Petri dish while the cutter is in the agar.

7. The operator now has the single spore resting on the top of a disc of agar within the cutter, which has been raised above the Petri dish. He has made sure that there is only one spore on the disc, and at this point has only to transfer the disc to a Petri dish or test tube of a suitable medium to complete the isolation. It has been found convenient to do this by lowering the biscuit cutter into sterile agar, thus forcing the disc containing the spore out through the top of the cutter (Fig. 1, B). The disc can then easily be removed by a sterile microspatula after the cutter is again raised above the Petri dish.

8. As a final step the cutter is flamed to sterilize it for the next isolation. This is easily done without removing it from the nosepiece of the microscope.

The device described above has distinct limitations and certain advantages that it may be well to outline briefly. The principal disadvantage of the method as a whole, as with the La Rue and Keitt methods, lies in the fact that the spore to be isolated must be on jelled medium and separated from other spores by a distance approximating the diameter of a low-power field. For this reason the method is not at all adapted, for example, to the removing of a sporidium from a promycelium, for which the Dickinson[3] and Hanna[4] techniques are so well suited. The advantages of the method lie in the fact that rapid isolations can be made with absolute precision. The cutter is made to circumscribe the spore with the mechanical precision inherent to the La Rue method and yet is adapted to lifting out the agar disc somewhat in the manner described by Sass,[5] so that the agar containing the spore can be cut off with no danger of the "spatula" touching the surrounding agar. As with the La Rue method, time is saved by the fact that it is not necessary to mark the place of the spore.

The cost of the brass plug used to hold the "biscuit cutter" should not exceed $3.50,[6] or might be even less if made from a discarded microscope objective. Extreme precision is not necessary in making the plug owing to the fact that the centering of the cutter is readily accomplished by bending the supporting wire after the device has been screwed into the microscope.—**EDMUND B. LAMBERT**, Bureau of Plant Industry, Washington, D. C.

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[3] Dickinson, S. The technique of isolation in microbiology. Phytopath. 23: 357-367. 1933.

[4] Hanna, W. F. A simple apparatus for isolating single spores. Phytopath. 18: 1017-1021. 1928.

[5] Sass, J. E. The cytological basis for homothallism and heterothallism in the Agaricaceae. Amer. Jour. Bot. 16: 663-701. 1929.

Water Damage to a Citrus Relative, Fortunella margarita.—Following the heavy rains of March, 1938, a severe cracking was noted in the rinds of kumquats, *Fortunella margarita,* a citrus relative growing at the Citrus Experiment Station. The cracks generally extended vertically from the stylar end to the calyx end of the fruit and in depth ultimately completely ruptured the rind to the pulp. Occasionally, the cracks extended in other directions (Fig. 1).

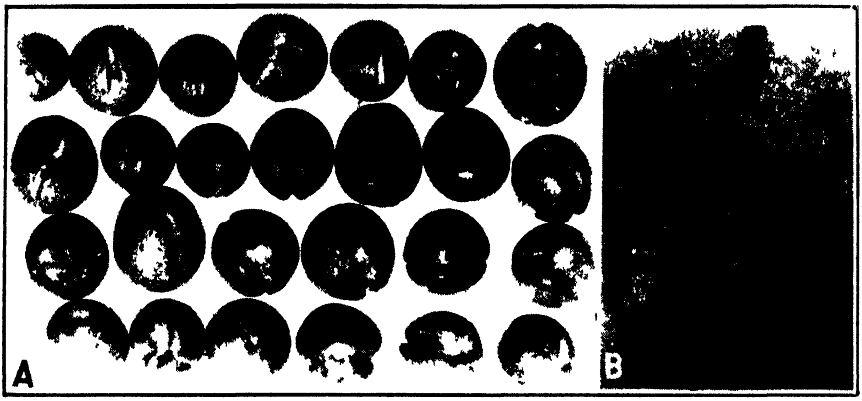

FIG. 1. A. Water damage to fruits of *Fortunella margarita.* × ½. B. Surface view of initial stages of water damage showing microscopic cracks. × 40.

As in the water-spot trouble of navel oranges the first evidence of injury was the presence of microscopic cracks or checks in the cuticle and underlying cells of the rind. The injuries soon became covered by dark-colored fungi, mostly *Cladosporium* sp. Several cracked fruit dropped.

The injuries were apparently attributable to the imbibitional and osmotic intake of rain water, which caused the fruits to swell and eventually split. To determine the possible relation of water to the injury, 15 leafy twigs bearing a total of 119 sound kumquat fruits were placed in a rain chamber for 68 hours. At the end of that period 85 of the 119 fruits, or 71.4 per cent, developed macroscopically visible cracks of various stages of severity. The more advanced the stage of maturity, as judged by color, the more severe were the ruptures.

Kumquat fruits and Washington Navel oranges were collected on March 31, 1938, placed in double cellophane bags, and frozen at 0° F. The juices were then expressed by means of a hydraulic press employing a pressure of 25,000 pounds per square inch. On the juices thus obtained were estimated total sugars after inversion and the depression in freezing point (Table 1).

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[6] To date, the only company asked to bid on these plugs is the Spencer Lens Company, who will furnish them for $3.50 on individual order, or $1.40 each in lots of 25.

TABLE 1.—*Estimated total sugars from fruit and rind, respectively, of kumquat and Washington Navel orange after inversion and the depression in the freezing point*

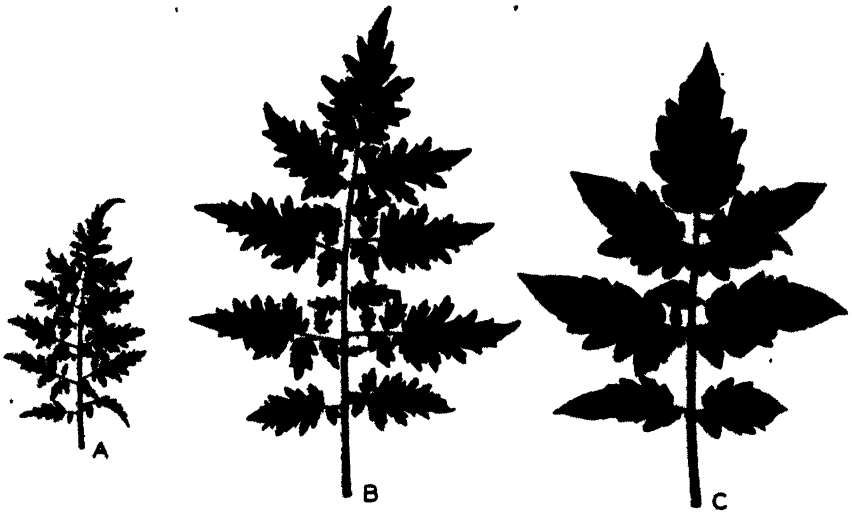
Juice pressed from fruit (after freezing)	Fehling's reduction after inversion (as per cent glucose)	Freezing point depression (Δ° C.)	Osmotic values in atmospheres
Whole kumquat fruit	16.14	3.04	36.47
Rind of kumquat fruit	17.84	2.9775	35.72
Whole Washington Navel fruit	11.72	1.6026	19.27
Rind of Washington Navel fruit	15.54	2.725	32.71

Fruit collected May 5, 1938, was peeled to remove the flavedo, then frozen, and the juices recovered by hydraulic pressure. Analysis showed 35 per cent more pectin in the kumquat sap than in that of Washington Navel.

These factors explain in part the greater susceptibility of kumquats than Riverside Washington Navel oranges to water damage. Navels grown in the vicinity of Riverside are much less susceptible to water damage than those grown eastern Los Angeles County. The possible causes of this greater resistance to water damage is suggested by the above comparison with kumquats. A comparison of Washington Navel oranges from several localities will be made during the coming navel season.—L. J. KLOTZ, Citrus Experiment Station, Riverside, California.

The Chilean Tomato, Lycopersicon chilense, as a Possible Source of Disease Resistance.—In past attempts to secure resistance to various diseases, no valid and distinct species appears to have been crossed with the cultivated tomato, *Lycopersicon esculentum* Mill., except the closely related Red Currant tomato, *L. pimpinellifolium* Mill. Recently, a dried fruit of the desert-inhabiting, perennial Chilean tomato, *L. chilense* Dun., was secured from sheet 5589 of the Gray Herbarium through the kindness of I. M. Johnston, who had collected it in the vicinity of Paposo, Chile, on December 8, 1925. The seeds in this fruit were about 12 years old, but one of them proved viable. The seedling grown from it showed the characteristics of the species. It had finely divided fern-like leaves (Fig. 1, A), and conspicuous stipules and floral bracts. It bore greenish, hairy fruits, that became creamy white and about 1 cm. in diameter when mature.

Recently the F_1 hybrid *Lycopersicon esculentum* × *L. chilense* was produced. The hybrid plants are extremely vigorous in growth. They have leaves (Fig. 1, B) that are intermediate in character between those of the two parent species, being more finely cut than ordinary tomato foliage (Fig. 1, C), but less finely cut than the foliage of *L. chilense* (Fig 1, A).



Photograph by J. A. Carlile

FIG. 1. Leaves of *Lycopersicon chilense* (A), *L. esculentum* (C), and the F_1 hybrid *L. esculentum* × *L. chilense* (B). Leaves comparable in age, and photographed at same scale.

Stipules and floral bracts are conspicuous in the hybrid, as in *L. chilense,* although they are absent from *L. esculentum.*

The species *Lycopersicon chilense* has been known to botanists for some 87 years, but, apparently, has not been utilized by horticulturists in the past. The purpose of this note is to indicate its present availability. The writer's interest in this species is concerned with the possibility of securing some form of resistance to the mosaic disease of tomato that is caused by tobacco-mosaic virus. It is not yet known whether any useful sort of resistance to this disease can be derived from the hybrid, but an attempt will be made to secure additional generations for further studies.—FRANCIS O. HOLMES, Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

Paradichlorbenzol, an Eradicant Fungicide, Effective Against Downy Mildew of Tobacco.—Potentialities of eradicant fungicides for increased use in plant-disease control have been pointed out by Keitt and associates.[1, 2] The development of rapidly acting, gaseous eradicant fungicides as substitutes for relatively inefficient aqueous protectant fungicides has been developed on a practical basis both in Australia and in the United States for the control of downy mildew of tobacco. A review of this work, to date,

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[1] Keitt, G. W., and E. E. Wilson. A possible reorientation of aims and methods for apple scab control. (Abstract) Phytopath. 17: 45. 1927.

[2] Keitt, G. W., and D. H. Palmiter. Potentialities of eradicant fungicides for combating apple scab and some other plant diseases. Jour. Agr. Res. [U. S.] 55: 397–437. 1937.

is reported by McLean and co-workers.[3] Vapors of other hydrocarbons have also been shown by Wolf, *et al.*,[4] to possess fungicidal properties. By cooperative experiments conducted by Mr. W. M. Lunn, Pee Dee Experiment Station, Florence, South Carolina, Mr. E. G. Moss, Oxford Experiment Station, Oxford, North Carolina, and the writers, at Chatham, Virginia, the eradicant fungicidal properties of gaseous paradichlorbenzol were demonstrated.

The results of seed-bed experiments during the downy-mildew epidemic of 1938, have shown that the vapors of paradichlorbenzol possess fungistatic, fungicidal, and phytocidal properties, depending upon the methods of application and amounts used. Work of like nature has been reported by Clayton.[5] Repeated nightly applications of 28 grams of crystalline paradichlorbenzol were found to be fungistatically equivalent to 25 ml. of liquid benzol in seed beds 4 sq. yd. in area and covered with cotton sheeting having a warp and woof of 64 threads per inch and a weight of 1 pound per 2.68 sq yd. Repeated experiments, however, showed the necessity of placing the crystals of paradichlorbenzol above the plants on suitable netting evapo-

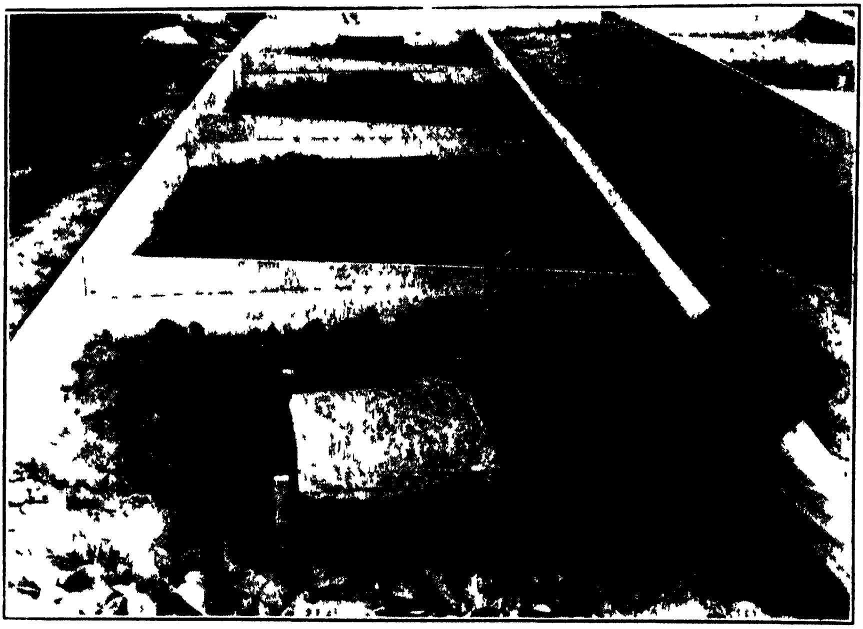

FIG. 1. Tobacco seed beds showing technic of paradichlorbenzol fumigation in the control of downy mildew. Seed bed covers were used only at night. Chatham, Va., 1938.

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[3] McLean, Ruth, F. A. Wolf, F. R. Darkis, and P. M. Gross. Control of downy mildew of tobacco by vapors of benzol and of other organic substances. Phytopath. 27: 982–991. 1937.

[4] Wolf, F. A., J. A. Pinckard, F. R. Darkis, Ruth McLean, and P. M. Gross. Field studies on concentration of benzol vapors as used to control downy mildew of tobacco. Phytopath. 29: 103–120. 1939.

[5] Clayton, E. E. Paradichlorbenzene as a control for blue-mold disease of tobacco. Science (n.s.) 88: 56. 1938.

FIG. 2. A. Fungicidal effect of 112 g. of paradichlorbenzol for one 12-hour period, using 18-inch square net evaporators. B. Phytocidal action of 453 g. of paradichlorbenzol using 4-sq.-yd. net evaporator on a 4-sq.-yd. seed bed.

FIG. 3. Section of 4-sq.-yd. tobacco seed bed, which received 225 g. of paradichlorbenzol distributed on net above plants for a 12-hour period. The "burned out" lesions caused by the fungicidal action of paradichlorbenzol upon *Peronospora tabacina* Adam. are shown.

rators protected from the weather (Fig. 1). The usual muslin sheeting as used on tobacco seed beds proved ineffective in retaining the heavy vapors and in preventing infection.

Eradicant fungicidal vapor concentrations were obtained in seed beds, 4 sq. yd. in area, when 112 g. of crystalline paradichlorbenzol was spread on net evaporators 18 in. square and treated as mentioned above (Fig. 2), although only one treatment of 12 hours was given the seed bed in each of 3 different experiments. By increasing the concentration of paradichlorbenzol to 225 g. spread over a net evaporator equal in area to the seed bed, phytocidal concentrations were approached. Fig. 3 shows the "burning out" effect of the vapor. In these experiments, the plants turned slightly yellow and doubtless suffered slight injury from the vapors, although they recovered after a few days. Grasses and weeds in the seed beds were seriously injured by the vapors.

Phytocidal vapor concentrations were obtained in seed beds, 4 sq. yd. in area, after one treatment with 453 g. of paradichlorbenzol (Fig. 2). In this experiment a net evaporator equal in area to the seed bed was used, together with a gas-proof or water-proof cover. Maximum temperatures during all of the experiments were less than 75° F.

In presenting these preliminary observations it is the writers' intention to suggest the possibilities of quick-acting vapors in the development of eradicant fungicides.—J. A. PINCKARD, Virginia Agricultural Experiment Station, Tobacco Research Laboratory, Chatham, Va., and RUTH MCLEAN, Duke University, Durham, N. C.

BOOK REVIEW

MARTIN, JOSEPH P. *Sugar Cane Diseases in Hawaii.* 295 p., 150 figs., with 13 colored plates. $8.50. Advertiser Publishing Company, Honolulu, Hawaii, U. S. A. 1938.

This work would seem to be another step forward in plant pathology, inasmuch as it is the product of increased specialization, which has led to more detailed knowledge. The author has specialized in the pathology of sugar cane throughout his whole career. His experience has not been confined to Hawaii, but has been gathered from extensive travels and conferences with the pathologists of other sugar-cane countries. Such travel and conference facilities are rarely afforded by usual research agencies and were chiefly possible in this case because of the unusually alert direction of intelligent and progressive capital, as represented by the Hawaiian Sugar Planters' Association.

The writer of this review, formerly engaged in sugar-cane pathology, can appreciate probably more than the casual reader the great amount of precise and informative subject matter contained in this work. Martin has had personal contact with all of the sugar-cane diseases described, and in most cases has actively engaged in research upon them. The descriptions and research results are, therefore, presented with intimate knowledge of the subject matter.

The work has a routine opening chapter on plant diseases in general, and a fine chapter on the value of plant quarantine for the exclusion of foreign sugar-cane diseases, particularly applicable to an island area such as the Hawaiian group. Descriptions, symptoms, extent of injuries, varietal resistance and susceptibility, causal agents, and control methods are presented in detail for diseases classified as to plant parts affected, such as leaf, stalk, and root diseases.

The chapter of 14 pages on nutritional diseases of sugar cane is especially valuable inasmuch as it was the work of Martin himself that showed the distinctive symptoms of a number of such nutritional deficiencies.

The chapter on the anatomy of the sugar-cane plant is also outstanding and well illustrated with diagrammatic drawings and photomicrographs.

There are chapters on malgrowths, animal, chemical, and mechanical injuries and the effects of such natural elements as lightning, sunburn, and wind effects upon sugar cane. In these chapters in particular the value of an agricultural research institution specializing on a single crop is evidenced, since association with investigators in other sciences on the same crop has advanced the correlation of symptoms with causal agents other than microorganisms.

One could be slightly critical of some of the type sizes and forms selected for such uses as chapter headings and certain divisions of the text. As an example, the type used in the table of contents is unnecessarily large, requiring 8 pages for presentation. The index, possibly more important to the reader, has an equally readable type and requires only 4½ pages for presentation. There also seems to be an excessive use of parentheses as a substitute for ordinary punctuation. These ideas of the reviewer, however, may be hypercritical.

The statement on page 2, ". . . recently several viruses have been shown by Stanley (145) to be caused by proteins" might possibly read, "Several infectious viruses have been shown by Stanley to be proteins, apparently of an autogenous nature." Such minor defects, if, indeed, they be defects, are infinitesimal as compared to the material value of the knowledge presented.

The colored plates, which include examples of the most common nutritional-deficiency symptoms, add substantially to the value of the work. The 150 additional figures, all well presented, are highly informative and show great detail.

The price of the book may seem high unless the specialized character of the work and the cost of the colored plates are considered. Detailed scientific knowledge is now being more widely appreciated as giving direct financial returns, and on such a basis the book is many times more valuable than the selling price.—ATHERTON LEE, Director, Puerto Rico Experiment Station, U. S. Department of Agriculture.

COLCHICINE IN THE PREVENTION, INHIBITION, AND DEATH OF PLANT TUMORS

NELLIE A. BROWN

(Accepted for publication October 31, 1938)

The effect of colchicine on malignant tumors of animals has been tested by Dustin (1), Amorosa (2), and other pathologists who worked largely with mice tumors. When the experimental animals were injected with colchicine they observed a collapse of cells and an inhibition of further tumor growth. In some experiments there was complete regression of tumors in 2 to 8 weeks.

László Havas (5) in his work with root tips and coleoptiles of wheat seedlings, in 1937, showed that colchicine produced a bulbous hypertrophy. The cytological examination of these swellings indicated that the mitotic figures closely resembled those found in the cells of the organs and tumors of animals treated with colchicine. This led him to test the effect of colchicine on the growth of tumors in plants (4). He found that a 1 to 10,000 aqueous solution introduced through a cut in the stems of tomato plants inhibited the growth of the tumors in plants inoculated with *Bacterium tumefaciens*. The inhibition was demonstrated in the reduction in size and number of the growths over the controls. From his experiments he suggested that colchicine inhibited the growth of plant tumors through the alteration of plant hormones.

According to his published report, Havas used only tomato plants for his tests on inhibition of tumorous growth with colchicine. The writer has attempted with a number of plant species not only to inhibit tumorous growths by means of colchicine but to prevent their formation and also to kill tumors already formed.

The *Bacterium tumefaciens* cultures used in the production of the plant galls were of the same strain that had been sent by the writer to Dr. Havas to use in his work. It is a very virulent strain and with it tumors were produced in 100 per cent of the plants not treated with colchicine (controls).

Tests were made with colchicine on 8 species of plants including *Chrysanthemum frutescens* L. (Paris daisy), *Tagetes patula* (French marigold), *Mirabilis jalapa* L. (four-o'clock), *Bryophyllum pinnatum* Kurz, *Kalanchoe daigremontiana, Nicotiana glauca* Graham (wild tobacco), *Phaseolus vulgaris* L. (bean), and *Lycopersicum esculentum* Mill. (tomato).

I. EXPERIMENTS TO PREVENT THE FORMATION OF PLANT TUMORS

A. By injecting inoculated plants with colchicine.

B. By inserting a lip of the basal stem in a solution of colchicine for several hours before inoculating with *Bacterium tumefaciens*.

C. By smearing with colchicine the area inoculated with (1) *Bacterium tumefaciens* or (2) indoleacetic acid in lanolin.

[VOLUME 29, NUMBER 3, MARCH, 1939]

A. *By Injecting the Inoculated Plants with Colchicine.* Young stems of Paris daisy, marigold, kidney bean, tomato, and *Kalanchoe* plants were inoculated with the crown-gall organism, *Bacterium tumefaciens,* immediately after which a 2 per cent solution of colchicine was injected hypodermically 1 to 1.5 cm. below the point of inoculation and in the region of the vessels occurring in a line with those receiving the infectious organism. The amount of solution injected was 0.2 to 0.5 cc. Usually, not all the solution injected was retained by the plant.

The appearance of the gall in the injected stems was delayed for several weeks after that of the controls, but in the majority of cases an overgrowth appeared that grew slowly but never reached the size of the controls. In a total of 61 plants inoculated and then injected with colchicine, tumors were prevented from forming in only 9 plants. This number included 2 daisy plants out of 15 treated, 4 marigolds out of 18, 1 tomato plant out of 14, 2 bean plants out of 7, and 0 *Kalanchoe* of the 7 plants treated. The controls, inoculated, but not injected with colchicine produced galls in all cases. The colchicine had a dwarfing effect on both the marigold and tomato plants, the topmost leaves of which became wrinkled and looked like those on plants affected with mosaic. In 3 weeks, however, most of the tomato plants outgrew this condition; but all, except a few of the marigolds, retained a stunted appearance. Injected colchicine, although somewhat inhibiting gall development, did not prevent their formation except in the 9 instances mentioned.

B. *By Inserting a Lip of the Basal Stem in a Solution of Colchicine for Several Hours Before Inoculating.* The method of treatment was as follows: An upward cut was made at the base of the stem and the lip inserted into a vial containing colchicine in varying concentrations from 0.06 to 2 per cent. After 4 or 5 hours, the vial was removed and the upper part of the stem was then inoculated with *Bacterium tumefaciens.* The plants treated in this manner were tomato, bean, Paris daisy, and *Kalanchoe daigremontiana.*

In 4 hours the tomato plants, for example, took up from 1 to 2.5 cc. of the solution from the vials. The effect on the tomato plants was a mottling of the upper leaves and a withering of some. This effect was noticeable in 2 to 5 days, depending on the concentration of the colchicine solution. There was no darkening of the stems. Within 6 weeks the mottling had disappeared and the 30 colchicine-treated plants were in a healthy condition. Their galls, however, were as large as those of the controls, with the exception that the treatment with the 2 per cent solution of colchicine resulted in much smaller galls than those on the controls.

Within the very low concentrations, there was no delay in the gall formation, but the 0.5, 1, and 2 per cent solutions delayed the appearance of the tumors over that of the controls in tomato and bean plants, but, in the end, galls appeared on all the plants treated.

Burpee's stringless bean plants, treated the same as the tomato stems, took up 1.7 to 3.4 cc. of the colchicine solution in 4 hours. The effect of the colchicine was more marked on the bean than on the tomato plants, but the time of its appearance was almost the same. Most of the bean leaves became mottled, but there was little or no withering. Some of the leaves dropped off and a few of the stems at the point of introduction turned brown. Later, the plants recovered, grew well, and in a month small galls had developed on all the 24 treated, as well as on the control plants. In 2 months the plants treated with 0.06 and those treated with 0.12 per cent solution had galls averaging 2.5 cm. by 2 cm. These were larger than the controls, which averaged 2 cm. by 1 cm. Those in the 2 per cent solution were approximately 0.5 cm. in diameter, much smaller than the control galls.

When kidney-bean stems were cut at the base and the lip inserted in 0.5 per cent solution of colchicine for 24 hours, the effect on the plant was rather severe. The amount of colchicine taken up in 24 hours was 0.8 to 1.7 cc. In 1 day a few spots showed on the upper leaves; in 6 days the upper leaves were brittle, spotted, and a few were dead. The tips of the stems were more or less stunted and did not shoot out like the controls. Later the plants branched and more or less recovered. In 2 months galls one-third the size of the controls had formed on 4 of the 6 treated plants. There was prevention of tumor formation in only 2 plants.

Kalanchoe diagremontiana stems were treated in a similar manner with 1 per cent colchicine for 5 hours in one test and for 24 hours in another, following which the upper stems were inoculated with *Bacterium tumefaciens*. The stems took up from 1 to 2 cc. of the solution. Tumors formed on each of the 14 treated plants, and in 2 months they were as large as the controls. The plants stood up well under the shock of the colchicine.

Plants of Paris daisy, given the same type of treatment, took up 0.5 to 0.8 cc. of 0.5 per cent colchicine in 4 hours. In 1 month there was no indication of gall formation on the 5 plants thus treated, whereas the nontreated plants had galls 1.5 cm. in diameter. In a little over 2 months, 2 of the treated plants developed galls, although they were much smaller than those on the control plants. The remaining 3 treated plants continued free from tumor formation.

Tests were made with tomato, bean, *Kalanchoe,* and daisy, in which the stems were inoculated before inserting the lip of the stem in colchicine. Tumor formation was not prevented by this method.

As a result of the basal lip treatment, only 5 plants did not form tumors in a total of 84 trials. These included 3 daisy, one of which is shown in figure 2, C–Ca, and 2 bean plants. Throughout the experiment care was taken to inoculate the stem on the side of the slit portion of the stem. Figure 2, D, shows the control gall of figure 2, C–Ca.

C. *By Smearing with Colchicine the Area Inoculated with (1) Bacterium tumefaciens or (2) Indoleacetic Acid in Lanolin.* Young bean stems, inoculated at the side with *Bacterium tumefaciens* and the inoculated area smeared over with 3 per cent colchicine in lanolin, produced galls in all 16 plants treated. The tumors were delayed in appearing, but grew to more than half the size of the controls.

Young bean stems were smeared at the side with 3 per cent indoleacetic acid in lanolin (3), followed by 3 per cent colchicine in lanolin over the same area. Small outgrowths developed in the seven plants treated, but they were smaller than the outgrowths produced by indoleacetic acid-lanolin smeared on bean stems as controls.

Kidney bean stems were decapitated and smeared with a 3 per cent indoleacetic acid-lanolin mixture followed by a 3 per cent colchicine-lanolin mixture. Of 17 bean plants treated, 12 developed galls that were smaller than the controls.

Nineteen young tomato plants were smeared on the upper stems with 3 per cent indoleacetic acid in lanolin; this area was then brushed with a 2 per cent colchicine solution. In a month and a half the colchicine-treated plants had galls half the size of the indole controls.

The upper stems of 12 young tomato plants were smeared with 3 per cent indoleacetic acid-lanolin mixture, then the area below was injected with 2 per cent colchicine solution. In 1 month nodulated galls formed on all the colchicine-treated plants. They grew slowly and in 3 months had reached the size of and looked like the control galls.

From these experiments it is apparent that colchicine in lanolin, smeared over inoculations made with *Bacterium tumefaciens* or indoleacetic acid in lanolin, did not prevent outgrowths from forming. In another section of this paper it will be shown that colchicine in lanolin smeared over bacterial tumors neither killed nor inhibited their further growth. The failure of colchicine in these cases may have been due to lack of solubility of the colchicine in lanolin.

II. EXPERIMENTS WITH COLCHICINE EITHER TO KILL OR PREVENT FURTHER DEVELOPMENT OF PLANT TUMORS

A. By brushing bacterial tumors with colchicine solutions.
B. By brushing indoleacetic acid tumors with colchicine solutions.
C. By injecting bacterial tumors with colchicine solutions.
D. By brushing bacterial tumors with a colchicine-lanolin mixture.
E. By inserting galled stems in a solution of colchicine, rooting in water, and then planting in soil.

This series of experiments proved to be highly successful in most of the plants tested.

A. *By Brushing Bacterial Tumors with Colchicine Solutions. Bacterium tumefaciens* tumors 1.5 to 2.5 cm. in diameter on 5 Paris daisy plants were brushed once with 0.5 per cent colchicine solution applied with a small camel-hair brush. The tumors increased in size and the surfaces looked the same for 10 days following the brushing. In 16 days growth had stopped and the galls began to darken. In 40 days all 5 were somewhat shrunken and black. In 10 weeks after brushing, the galls were dead but the tissue above and below them remained sound. The plants continued in a flourishing condition and later bore flowers. In 4 months from the beginning of

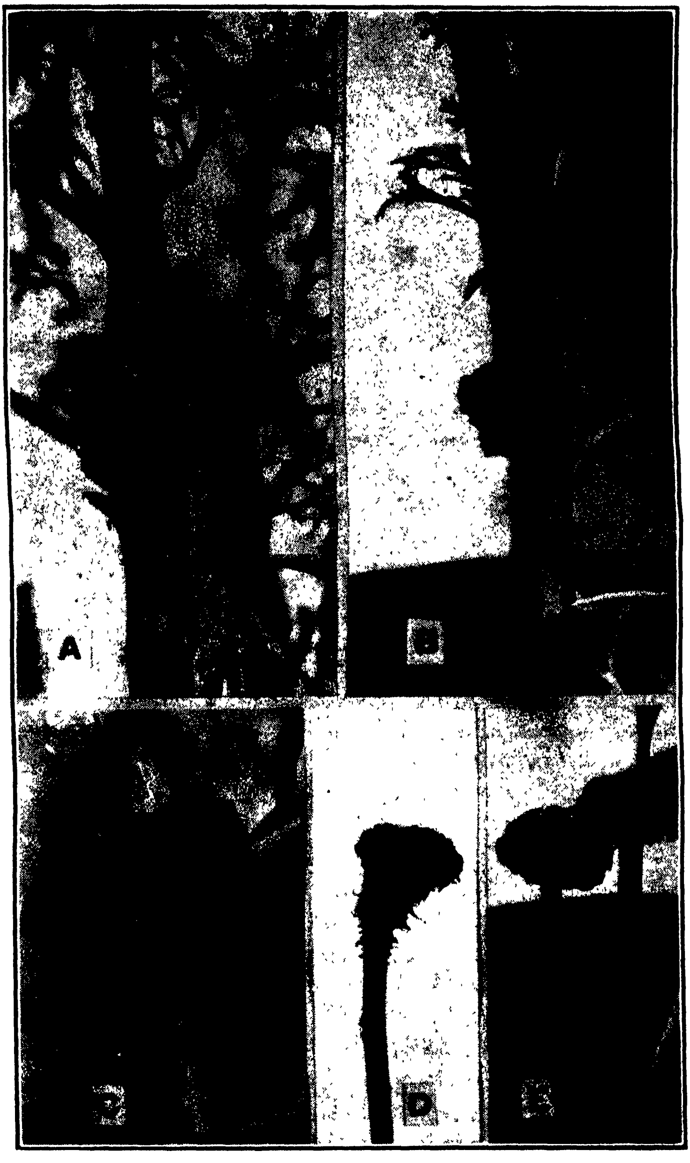

FIG. 1. The killing effect of colchicine on *Bacterium tumefaciens* galls. A. Gall killed by brushing once with 2 per cent colchicine. Inoculated daisy stem with *Bact. tumefaciens* November 23, 1937. Brushed the resultant galls with 2 per cent colchicine solution January 21, 1938. Photographed March 8, 1938. Gall dead, plant alive. B. Gall killed by injecting it with 2 per cent colchicine. Inoculated daisy stem November 23, 1937. Injected the resultant galls with 2 per cent colchicine solution January 21, 1938. Photographed March 8, 1938. Gall dead, plant alive. C. Control gall for A and B. Daisy stem inoculated November 23, 1937, with *Bact. tumefaciens*. Photographed March 8, 1938. D. Four-o'clock gall killed by brushing once with 2 per cent colchicine. Inoculated the cut-off stem April 23, 1938. Brushed the resultant gall with 2 per cent colchicine solution May 24, 1938. Photographed June 4, 1938. Gall dead, plant alive. E. Control of D. Four-o'clock stems cut off and inoculated with *Bact. tumefaciens* April 23, 1938. Photographed June 4, 1938. All natural size. The daisy plants on which the dead galls occur were alive and blossoming May 20, 1938.

the experiment with colchicine the control galls were 3.5 to 4 cm. in diameter and still in good condition.

Colchicine, 0.5 per cent, also was brushed on bacterial tumors of tomato plants, but no death of the tumors ensued. When a 2 per cent solution was brushed on other tomato tumors of bacterial origin, a few of the surface nodules of the tumor became brown; little further growth took place, although the galls remained alive. At the end of 6 weeks the control tumors were more than double the size of the treated ones.

In another experiment young Paris daisy stems inoculated with *Bacterium tumefaciens* for nearly 2 months had tumors 2.5 to 3 cm. in diameter. Fifteen tumors were brushed with 2 per cent colchicine. They continued to grow for a week to 10 days, then began to shrink slightly, and shortly afterward dark areas appeared, which spread slowly over the whole outgrowth. Within 2 months after brushing all 15 tumors were dead (Fig. 1, A). The plants, however, continued to grow and, 2 months after the death of the tumors, were still in good condition and blossoming. Several of the stems bearing dead galls were split apart and the stem tissue above and below the galls was found alive. One of these split stems is shown in figure 2, A. The size of the control tumors is shown in figure 1, C.

The above experiment took place in the winter. It was repeated in the spring and summer, using daisy tumors 1 to 2 cm. in diameter. A difference was noted in the earlier death of the galls brushed during the hot weather. They began to darken in a week or 10 days after brushing and, in some cases, died within 2 or 3 weeks. Occasionally, some galls did not grow perceptibly after brushing, but, usually, development continued for a few days to a week or 10 days. After that, they began to shrink, then the surfaces to darken; the whole tumor blackened and died. High temperatures and high humidity usually hastened the darkening and the collapse. The dead galls usually dried and were corky; they did not rot and rarely were attacked by a fungus while they were dying. This statement can be applied also to the dead galls of the various species. Of a total number of 101 Paris daisy galls brushed with 2 per cent colchicine, all died but 10. The controls were often double the size of the dead galls at the time of the complete collapse. None of the daisy plants was killed by brushing the galls with 2 per cent colchicine, nor did the subsequent growth and development appear to be interfered with.

Marigold tumors succumbed very readily when brushed with colchicine solutions. In one test 40 *Bacterium tumefaciens* galls, growing at the side of upper stems, were brushed with a 2 per cent colchicine solution. The galls were 1 to 1.5 cm. in diameter and growing rapidly. Growth continued for a week before they began to shrink; 3 days later the control galls were 3 times the size of the treated galls; 15 days from the time of brushing, the smaller galls were dry and dead, the larger ones dark and shriveling but still alive. In 6 weeks from the time of brushing, 35 of the 40 tumors were dead; the remaining 5 did not succumb later.

In another test with 82 marigolds in which the tumors were 2 cm. or a

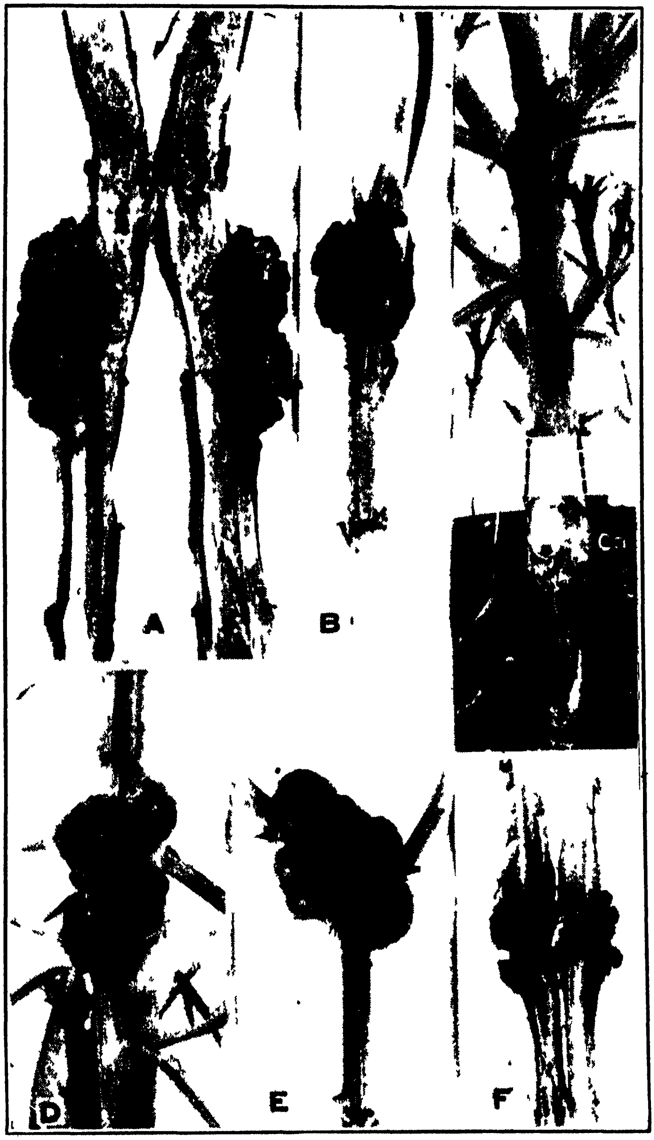

FIG. 2. A. Split stem of Paris daisy showing dead gall and stem alive. Inoculated stem with *Bacterium tumefaciens* November 23, 1937. Brushed gall once with 2 per cent colchicine solution January 21, 1938. The plant was alive and blooming when photographed May 24, 1938. Control of A is shown in Fig. 1, C. B. Marigold stem inoculated with *Bact. tumefaciens* June 10, 1938. Gall brushed once with 2 per cent colchicine solution June 28, 1938. Photographed July 28, 1938. Gall dead, stem alive. C–Ca. Stem of Paris daisy slit at base May 5, 1938, and triangular slit inserted in a vial of a one-half of 1 per cent colchicine solution for 4 hours, then upper stem inoculated with *Bact. tumefaciens*. No gall formed at inoculated place. Photographed June 13, 1938. D. Control of C–Ca. Daisy stem inoculated with *Bact. tumefaciens* May 5, 1938. Photographed June 13, 1938. E. Control of B and F. Marigold stem inoculated with *Bact. tumefaciens* June 10, 1938. Photographed July 28, 1938. F. A split stem of marigold showing dead gall and live stem. Inoculated stem with *Bact. tumefaciens* June 10, 1938. Brushed gall with 2 per cent colchicine June 28, 1938. Photographed July 28, 1938. All natural size.

little more in diameter they did not stop growing until 7 days after brushing with 2 per cent colchicine. However, in 15 to 28 days after brushing, all 32 tumors were dead (Fig. 2, B and F). One of the control tumors is shown in figure 2, E.

Nineteen other smaller marigold tumors, 4 to 8 mm. across, were brushed in the same way with 2 per cent colchicine. These 19 also continued to grow for a week before darkening, but all except 2 were dead in 13 to 21 days after brushing.

In the early fall 21 decapitated marigold plants with bacterial tumors 1 to 1.2 cm. in diameter were brushed with 2 per cent colchicine. Seven other bacterial galls of the same age and size on decapitated marigold plants were brushed with 0.5 per cent colchicine. The tumors of both sets continued to grow 8 to 9 days after brushing, then darkened, and all galls were dead 15 to 16 days after the colchicine treatment. Those galls brushed with the 0.5 per cent solution succumbed as readily as those brushed with 2 per cent colchicine. The control tumors continued to grow, reaching an average size of 2 cm. The plants with dead galls remained vigorous, branched below the decapitated portion and continued a healthy normal life.

In every case the marigold plants with dead galls continued to live and blossom until 2 months later, when they were discarded.

Eighteen bacterial galls, 8 to 12 mm. in diameter, 1 month old, on lopped stems of four-o'clock plants, *Mirabilis jalapa,* were brushed with 2 per cent colchicine solution. In 7 days 12 of them had blackened and in 11 days all were dead (Fig. 1, D). It was early summer and the plants had been held part of the time in a moist chamber, with probable consequent hastening of the effect of the colchicine. The control galls remained in good condition (Fig. 1, E) and continued to develop.

Bacterium tumefaciens galls, 1 to 1.5 cm. in diameter, on tomato plants, were brushed with 2 per cent colchicine solution. A slight darkening occurred in 7 days, and growth was inhibited in 7 of 10 tumors. After 6 weeks none had died and 3 were still growing slowly.

In another test 42 tomato galls, 1 to 1.2 cm. in diameter, were brushed with 2 per cent colchicine. There was a slight darkening of the nodular surfaces in 5 days. In 2 weeks, growth was inhibited in 6 galls; the others continued to grow for some time. After nearly 2 months none of the 42 galls had died, although the treatment had inhibited or dwarfed their development. They were $\frac{1}{4}$ to $\frac{1}{3}$ the size of the controls.

Six *Bacterium tumefaciens* galls 0.5 to 1 cm. in diameter on *Nicotiana glauca* were brushed with a 2 per cent solution of colchicine. The outgrowths continued to increase in size for 11 days, then a shrinking occurred and the surfaces became corky and dark colored. In 6 weeks two of the galls were dead and the rest dying.

Kalanchoe diagremontiana and *Bryophyllum pinnatum* plants had been inoculated nearly 2 months with *Bacterium tumefaciens* and galls 2 to 2.5 cm. in diameter had formed on the former and 7 to 13 mm. on the latter.

They were brushed at the same time with 2 per cent colchicine. Growth continued for 2 weeks in the *Kalanchoe* and somewhat longer in the *Bryophyllum* before there was retardation. Death, however, did not take place until 4 months after brushing the *Kalanchoe* galls and 5 months after brushing the *Bryophyllum* galls. The plants remained alive and grew until they were discarded 3 months after the tumors had died.

B. *By Brushing Indoleacetic Acid Tumors with Colchicine Solutions.* Twelve indoleacetic acid-lanolin tumors, approximately 0.2 cm. in diameter, produced at the side of young bean stems, were not destroyed by brushing with 2 per cent colchicine solution. Further development was stopped, however, and, except for some shrinking, the galls continued to look the same for 6 weeks. The controls grew to approximately 1 cm. in diameter in 2 weeks.

Nine indoleacetic acid-lanolin tumors, 0.5 to 1 cm. in diameter produced on decapitated bean stems, were brushed with a 2 per cent solution of colchicine. The galls were not killed but further growth was inhibited. The control galls continued to grow to a diameter of 2 cm. before the plants were discarded.

Indoleacetic acid-lanolin galls, on stems of *Nicotiana glauca,* for a time looked much like *Bacterium tumefaciens* galls. The largest was 0.5 cm. across at the time of brushing with 2 per cent colchicine. Further growth was inhibited after 8 to 10 days, but the galls, 8 in number, were still alive 6 weeks from the time of brushing.

Seven indoleacetic-lanolin galls on marigold stems 8 days old, and larger than those on the side of the bean or tobacco plants indicated above, were covered with masses of root primordia at the time of brushing with 2 per cent colchicine. There was inhibition of growth in 4 days, but death of the tumors did not ensue. The indoleacetic acid-lanolin controls continued to grow well for 3 weeks and attained a diameter of over 1 cm.

C. *By Injecting Bacterial Tumors with Colchicine Solutions.* When *Bacterium tumefaciens* galls on the Paris daisy were 1.5 to 2.5 cm. in diameter, they were injected hypodermically with a 0.5 per cent solution of colchicine. From 0.2 to 1 cc. was injected into the galls, but not all of it was retained. There was no trace of darkening of the tumor in the first week after the injections, but in 2 weeks dark areas were common. A few days later all the galls were black and had begun to shrivel. In 2 months, all of the 15 injected tumors were dead. When a 2 per cent solution of colchicine was injected into 7 daisy tumors of the same size, the galls continued to grow for 13 days, although dark areas appeared where the hypodermic needle entered the gall. In a month the injected tumors were shrunken and black and in 6 weeks all 7 were dead (Fig. 1, B). The plants continued to grow and bloom. One of the control tumors is shown in figure 1, C.

Galls on four-o'clock plants were killed in a much shorter time when a 2 per cent solution was injected. The four-o'clock stems were decapitated and the cut surfaces smeared with an agar culture of *Bacterium tumefaciens.*

In a month, galls had formed 8 to 12 mm. across. These received an injection of 2 per cent colchicine, 0.2 to 0.5 cc. in each gall. In 11 days the 6 injected galls were dead, while the control galls were alive and continued to grow.

D. *By Brushing Bacterial Tumors with a Colchicine-lanolin Mixture.* Two months after inoculating 10 daisy plants, the galls, 1.5 to 2 cm. in diameter, were brushed with 3 per cent colchicine-lanolin mixture, heated to facilitate its spreading over the tumor surfaces. The 10 tumors continued to increase in size for more than 10 weeks. Tumors of *Kalanchoe* and *Bryophyllum,* the same age as those on the daisies and nearly the same size, also were brushed with the liquefied colchicine-lanolin mixture. All the tumors were still growing 4 months later and at that time were 3 to 4 cm., the size of the controls.

E. *By Inserting Galled Stems in a Colchicine Solution, Rooting in Water, and then Planting in Soil.* Some *Kalanchoe* plants were subjected to a test that none of the other plants received. They had been inoculated with *Bacterium tumefaciens* a little over a month and the galls were 1 to 1.2 cm. in diameter. The stems of 4 plants were cut off at the base, brought to the laboratory and placed in bottles containing 0.5 per cent colchicine solution. At the end of 4 hours no appreciable amount had been taken up, so the plants were left in the solution for 18 hours, by which time they had absorbed 1.4 cc., 1.5 cc., 1.8 cc., and 2 cc. of the colchicine, respectively. From the colchicine they were changed to beakers of water and left for 10 days. By this time the stems were well rooted and roots were extending from the galls also. The 3 controls in water had fewer roots from their bases and none from the galls. All were planted in soil in the greenhouse and grew well for a time; but, in a month, the tumors on the treated plants began to blacken and 10 days later were dead. In another week the plants also were dead, while the controls remained healthy and continued to grow for 3 months or more.

In all probability, death of the treated galls does not result from any direct action of the colchicine on killing or inhibition through *Bacterium tumefaciens.* This view is supported by the fact that reisolations of the organism can be made from treated inhibited galls and also by the fact that the organism can apparently withstand relatively high concentrations of colchicine added to culture media. It seems more likely that death of the galls is to be explained by a differential response of tumor and normal tissue to colchicine, or that its action is in some way related to alteration of the growth substances, as suggested by Havas.

SUMMARY

Different methods of using colchicine were tried in attempting to prevent the inception of plant tumors, to inhibit growth that had begun, and to kill tumors already formed. Tumor formation was successfully prevented in only a few cases. It is possible that a still different technique would have been accompanied by greater success.

Brushing the surfaces of bacterial tumors was found to be an effective method for inhibiting further growth and, in time, killing the tumor. No more than one brushing was given. Of the total of 305 tumors, 239 died after being brushed with colchicine. Of the 49 tomato tumors included in this number, none succumbed to the treatment. Although the tumors were killed, other parts of the plant remained healthy and continued to live and function normally throughout the life span of the plant.

Brushing the surfaces of indoleacetic acid tumors with 2 per cent colchicine solution inhibited further growth but did not kill the tumors. Stronger solutions, which may be effective in killing indoleacetic acid tumors, have not been tested.

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THE EFFECT OF SOME SOIL FACTORS ON PENICILLIUM INJURY OF CORN SEEDLINGS[1]

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An actual estimate of the importance of the Penicillium seedling disease of corn would be difficult to make, but Koehler and Holbert (11) have said, "The fact that *Penicillium oxalicum* has frequently been isolated from corn ears and that pure-culture seed inoculations have caused striking reductions in stand and vigor as compared with check plots grown from similar but uninoculated seed, all grown under favorable field conditions, indicates that the disease may be of considerable importance."

Comparatively little work has been done on the effect of soil conditions on this disease. It is the purpose of this paper to report the result of some greenhouse investigations on: (a) the effect of soil conditions, especially moisture and soil texture, on the severity of Penicillium injury to corn seedlings; (b) partial protection offered wounded kernels by species of *Penicillium* against attack by *P. oxalicum* (Currie and Thom) from the soil; (c) nature of the injury produced by *P. oxalicum.*

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Studies on Penicillium injury to corn seedlings were first reported by Johann (8). In a later report on more comprehensive studies, Johann, Holbert, and Dickson (9) described the injury to seedlings grown from inoculated kernels as follows: "Symptoms of seedling injury caused by *P. oxalicum* are manifest in a yellowing of the leaves, a retardation in the growth of the plant, and in cases of severe infection, more or less complete drying of the leaves. In the last case, death usually occurs before the sixth leaf has unfolded. Such plants, as well as others less severely injured, show scarcely any rotting of the mesocotyls or root systems."

In the investigations here reported, greenhouse and field tests showed that *Penicillium oxalicum* caused severe injury to corn seedlings if the kernels were wounded and inoculated by immersion in a spore suspension or were wounded and planted in soil infested with the fungus; the stand was decreased, the vigor was impaired, and the height of the seedlings was greatly reduced. The injury was similar to that described by Johann. Often not more than 2 leaves were produced before the plant died; in many cases the plumule did not even break through the coleoptile. Seedlings from wounded, noninoculated kernels planted in sterile soil did not differ from those grown from sound kernels.

Although the Penicillium seedling blight in these studies was severe only when the pericarp was broken, Koehler (10) showed that in 200 seed samples in Illinois an average of 10 per cent of the kernels showed crown injury. When such injured but noninoculated kernels were planted in field or greenhouse, there was reduction in stand, vigor, and yield, presumably caused by infections from the soil. It is not known how often *P. oxalicum* is involved in the field in such infections.

MATERIAL AND METHODS

Open-pollinated yellow dent corn was used throughout these studies. Kernels were wounded by shaving off the pericarp from the end of the crown. Inoculation was obtained by immersing the kernels for a few minutes in a suspension of spores in sterile water. Soil to be inoculated was steamed without pressure 2 to 3 hours and cooled; corn meal on which the fungus had been growing 6 to 7 days was then mixed with it. The studies were confined for the most part to the greenhouse.

Each experiment was repeated several times, and the data presented are typical of the results obtained. It was felt that the average height of seedlings could be used as a measure of the amount of injury, since Koehler and Holbert (11) found that "the perfection of the field stand under any given conditions is in proportion to the degree of vigor of the seedlings. Weak seedlings usually develop into plants that are handicapped throughout their life period as compared with plants from strong seedlings.''

THE EFFECT OF SOIL MOISTURE

Preliminary experiments showed that at times the Penicillium seedling blight was not so severe in moist soil as in dry soil. Because wet soil did not consistently reduce the severity of the disease, it was felt that the effect of moisture may have been influenced by some additional factor, such as the physical condition of the soil. Consequently the effect of the manner of planting kernels in soil, as well as the effect of soil moisture, was studied.

The writer had observed that when wounded and inoculated kernels were pressed into close contact with very wet clay loam the degree of injury was less than when wounded and inoculated kernels were planted in loose wet or dry soil. To check this observation further, the following experiments were performed.

In duplicate plots of soil in the greenhouse bench, furrows 2 inches deep were dug 5 inches apart; the soil was wet to saturation. One hundred wounded and inoculated, and 100 sound noninoculated kernels were pushed into the soil with the wounded portion pressed close against the soil; 100 wounded and inoculated, and 100 sound and noninoculated kernels were placed on the surface of the soil in the furrow. The furrows were then covered with soil; the soil was wet to saturation and watered each day.

Another plot was planted in the same way, but the soil moisture was maintained at approximately 40–50 per cent of its moisture-holding capacity. In these experiments this soil is designated ''dry.''

The plants were measured 16 days after planting. The results presented in table 1 indicate that when wounded and inoculated kernels were pressed

TABLE 1.—*Effect of soil moisture on seedlings from wounded corn kernels inoculated with spores of Penicillium oxalicum and pressed into contact with the soil. One hundred kernels per plot*

Soil	Treatment of kernels	Manner of planting kernels	Percentage stand	Average height in centimeters
Dry	Sound Noninoculated	Pressed into soil	95	19.2
Dry	Wounded Inoculated	Pressed into soil	39	1.7
Dry	Sound Noninoculated	In loose soil	100	20.4
Dry	Wounded Inoculated	In loose soil	20	2.1
Wet	Sound Noninoculated	Pressed into soil	100	26.0
Wet	Wounded Inoculated	Pressed into soil	100	18.0
Wet	Sound Noninoculated	In loose soil	96	24.6
Wet	Wounded Inoculated	In loose soil	68	4.7

closely against wet soil, and were presumably in close contact with it, the severity of the disease was reduced. When wounded and inoculated kernels were similarly in contact with dry soil there was no apparent reduction in the degree of injury, nor was there any reduction in injury when wounded and inoculated kernels were planted in loose wet or loose dry soil.

To learn whether this effect of soil moisture was on the fungus or the host, kernels were planted on which the wound was produced by shaving one edge of the crown rather than removing the entire end. In one lot the kernels were wounded on the embryo side of the crown, inoculated, and pressed into wet surface soil with the embryo side uppermost; thus the wounded portion was not in close contact with the soil. Another lot was wounded on the side opposite the embryo, inoculated, and pressed into the wet soil with the embryo side up, and the wounded part of the kernel in contact with the soil. Both these lots and one lot of sound noninoculated kernels were then lightly covered with soil. A similar series was planted in dry soil at the same time. In all cases the living embryo was under the same conditions, namely, under a thin layer of soil; the fungus on the wounded part of the crown of the endosperm was pressed into contact with the soil in some lots, and under only a thin layer of soil in the others; any difference in the groups of plants would then be attributable to the effect on the fungus rather than the host, since the host was treated similarly in all lots, but the fungus on the wound was subjected to different conditions.

Table 2 shows that the results were similar to those obtained in the previous experiment in that when the fungus on the wound was pressed into wet soil there was a reduction in the amount of injury. Apparently the effect was on the fungus rather than the host. The suggestion occurs that this effect may lie in a decreased oxygen supply to the fungus in the wet soil compacted by the pressure put on the kernel. In this connection it may be noted that the writer had observed that *Penicillium oxalicum* grew well on

TABLE 2.—*Effect of soil moisture on vigor of Penicillium oxalicum on kernels of corn as indicated by the height of the seedlings grown from wounded inoculated kernels. One hundred kernels per plot*

Soil	Treatment of kernels	Treatment of fungus on wound	Percentage stand	Average height in centimeters
Dry	Wounded Noninoculated	Pressed into soil	100	30.0
Dry	Wounded Inoculated	Covered lightly	82	4.6
Dry	Wounded Inoculated	Pressed into soil	86	7.6
Wet	Wounded Noninoculated	Pressed into soil	100	36.4
Wet	Wounded Inoculated	Covered lightly	70	4.8
Wet	Wounded Inoculated	Pressed into soil	94	27.9

nutrient agar and in liquid nutrient media, but when hyphae were introduced into soft warm nutrient agar in test tubes and covered with a layer of agar, the growth of the fungus was very slow.

To determine the amount of soil moisture necessary to produce any reduction in the degree of injury to young diseased seedlings, a series of soils with different moisture contents was set up. Three 2-gallon pots of soil were brought to each of the following degrees of moisture content: 40, 60, and 80 per cent of the moisture-holding capacity, and saturation. In each series 25 wounded and inoculated kernels were pressed into the soil in one pot, 25 were planted loosely in another pot, and 25 sound and noninoculated kernels in a third were controls. The moisture content was maintained approximately by daily weighing and watering when necessary.

The results given in table 3 indicate that in soil with 60 per cent or less moisture there was no appreciable difference between plants grown from kernels planted in loose soil and those pressed into contact with the soil. Evidently, the soil must be very wet to have any effect.

TABLE 3.—*The severity of Penicillium injury to corn at different degrees of soil moisture, with some kernels in loose soil and others pressed into the soil*

Kernel	Manner of planting	Average height (cm.) Soil moisture maintained at:			
		40%	60%	80%	Saturation
Sound Noninoculated	Pressed into soil	13.2	14.7	17.7	19.4
Wounded Inoculated	In loose soil	1.7	1.6	3.5	4.4
Wounded Inoculated	Pressed into soil	1.4	2.7	9.2	14.9

PARTIAL PROTECTION TO CORN SEEDLINGS BY SPECIES OF PENICILLIUM AGAINST ATTACK FROM SOIL INFESTED WITH PENICILLIUM OXALICUM

In recent years considerable evidence has been accumulated to bring out the importance of antagonism among microorganisms, especially soil fungi. Fawcett (5) has said, "Nature does not work with pure cultures alone, but most frequently with associations." Arrillaga (1), Broadfoot (2), Fawcett (5), and Porter (12) have reviewed some of the pertinent papers on this subject. Koehler (10) has shown that *Penicillium notatum* protected corn kernels against fungus attack from field soil.

In the present studies the writer observed that when wounded kernels were inoculated with some isolates of *Penicillium* they were partially protected when planted in soil lightly inoculated with *Penicillium oxalicum.* Table 4 shows the protective action of *P. notatum* and *P.* spp. (Isolate K3), an undetermined species physiologically and morphologically different from *P. oxalicum* and *P. notatum.*

The degree of protection varied with the amount of inoculum in the soil,

TABLE 4.—*Partial protection offered corn seedlings by Penicillium notatum and Isolate K3 against attack from soil inoculated with P. oxalicum. One hundred kernels per plot*

Kernel	Treatment	Average height in centimeters
Sound	None	21.9
Wounded	None	8.6
Wounded	0.5 per cent Ethyl mercury phosphate	15.6
Wounded	Isolate K3	16.0
Wounded	*Penicillium notatum*	14.1
Wounded	*Penicillium oxalicum*	2.4

and the spore load of the protecting fungus on the kernels. One lot of 25 wounded kernels was inoculated with a spore suspension of K3 and planted in soil inoculated with *Penicillium oxalicum;* a second similar lot was planted in soil inoculated with half as much inoculum; a third lot was inoculated more heavily with K3 by applying a coating of dry spores to the wounded part of moistened kernels, and then planting in soil inoculated with *P. oxalicum,* as in the first lot. Plants from these lots were compared with those from wounded and noninoculated kernels planted in inoculated soil. A similar series was set up with *P. notatum* in place of K3. From the data in table 5 it is seen that the protection offered by *P. notatum* and K3 was increased with an increase in the spore load of these fungi on the kernels and with a decrease in amount of *P. oxalicum* in the soil.

TABLE 5.—*The effect of spore load of Penicillium notatum and K3 on kernels, and of the amount of inoculum of P. oxalicum in the soil on the degree of protection against attack of the kernels from the soil. Twenty-five kernels per plot*

Kernel	Inoculation	Soil inoculation	Average height in centimeters
Wounded	Isolate K3	*Penicillium oxalicum*	5.4
Wounded	Isolate K3	*Penicillium oxalicum* Half normal load	15.1
Wounded	Isolate K3 Heavy load	*Penicillium oxalicum*	[illegible]
Wounded	*Penicillium notatum*	*Penicillium oxalicum*	4.3
Wounded	*Penicillium notatum*	*Penicillium oxalicum* Half normal load	12.4
Wounded	*Penicillium notatum* Heavy load	*Penicillium oxalicum*	14.3
Nonwounded	None	*Penicillium oxalicum*	24.0
Wounded	None	*Penicillium oxalicum*	2.9
Wounded	None	*Penicillium oxalicum* Half normal load	8.9

Furthermore, when wounded kernels inoculated with Isolate K3 or *Penicillium notatum* were kept in moist chambers 24 hours before planting in soil inoculated with *P. oxalicum,* the degree of protection against attack by the latter was greater than when the kernels were planted immediately after inoculation (Table 6).

TABLE 6.—*Increased protection against infection of corn seedlings from soil infested with Penicillium oxalicum when the protecting fungi are given a time advantage. Twenty-five kernels per plot*

Kernel	Inoculation	Average height in centimeters
Nonwounded	None	24.0
Wounded	None	2.9
Wounded	Isolate K3; planted at once	5.4
Wounded	Isolate K3; planted 24 hours after inoculation	15.0
Wounded	*Penicillium notatum;* planted at once	4.3
Wounded	*Penicillium notatum;* planted 24 hours after inoculation	17.2

When wounded kernels were sterilized with $HgCl_2$ and then inoculated with Isolate K3 or *Penicillium notatum,* and grown on sterile sand in flasks or in sterilized or unsterilized field soil, there was no difference between seedlings grown from nonwounded or wounded noninoculated kernels and those from kernels wounded and inoculated with K3 or *P. notatum;* these two species were not injurious to corn seedlings.

To determine whether there was any antagonistic action between *Penicillium oxalicum* and these protecting fungi in culture, *P. oxalicum, P. notatum* and K3 were grown separately in autoclaved Richard's solution. After 7 days the solutions were filtered separately through a Berkfeld filter into sterile flasks. The solutions were then reinoculated with spore suspensions; the filtrates from all 3 fungi were inoculated with spores from all 3 fungi, respectively, in separate flasks. At no time for 2 weeks was there any difference in the rate of growth of any of the fungi on any of the filtrates as compared with growth on Richard's solution.

When this experiment was repeated with filtrates from cultures 2 weeks old, growth of all fungi on all filtrates was slower than growth in Richard's solution. This probably was caused by a decrease in available food in the filtrates after they had supported growth for 2 weeks, or by staling products in all filtrates.

When colonies of *Penicillium oxalicum* and K3 were grown side by side on potato dextrose agar in Petri dishes, the colonies often grew together. There seemed to be no antagonistic toxic substance produced by isolate K3 which would inhibit growth of *P. oxalicum.* Nor was there any antagonism between *P. notatum* and *P. oxalicum* on agar.

STUDIES ON THE NATURE OF INJURY

Studies on the nature of fungus injury to plants have been conducted over a long period of time. It has been suggested by various workers that such injury is caused by enzyme action on the middle lamella and cell walls, by oxalic acid, nitrites, aldehydes, and toxins.

The literature in this field of investigation has been reviewed by Brown (3), Higgins (6), White (13), and Wolf (14) among others. In a preliminary study on the nature of the injury caused by *Penicillium oxalicum* it was found by the writer that corn seedlings did not grow well in liquid culture media on which the fungus had been growing. The fungus was grown on Richard's solution for 2 weeks, and was then filtered off. The filtrate was sterilized by passage through a Berkfeld filter. Wounded surface-sterilized kernels were placed in sterile flasks on sterile cotton soaked in the filtrate. At the end of 2 weeks the seedlings were stunted, somewhat distorted, and resembled those grown from wounded inoculated kernels. There was no sign of fungus growth.

To see whether the fungus produced a similar toxic filtrate on corn kernels, it was grown 14 days on 25 surface-sterilized, wounded, living kernels. The kernels were then ground in a mortar, 100 cc. distilled water was added and the mixture allowed to stand 24 hours. The filtrate was sterilized as in the previous experiment. Filtrates were obtained in the same way from autoclaved inoculated and noninoculated kernels and from surface-sterilized noninoculated kernels 14 days after germination. Table 7 shows that the filtrates from autoclaved or living kernels inoculated with *Penicillium oxalicum* were toxic to seedlings, while filtrate from noninoculated kernels was not.

TABLE 7.—*Growth of corn seedlings on filtrates from Penicillium oxalicum, P. notatum, and K3. Fifteen kernels per plot*

Filtrate from:	Average height in centimeters
None. Sterile water	21.8
Sound, autoclaved kernels	23.1
Sound, noninoculated kernels 14 days after germination	22.0
Penicillium oxalicum on Richard's solution	3.1
Autoclaved kernels inoculated with *Penicillium oxalicum*	4.6
Living kernels inoculated with *Penicillium oxalicum*	6.7
Penicillium notatum on Richard's solution	20.1
Autoclaved kernels inoculated with *Penicillium notatum*	19.2
Living kernels inoculated with *Penicillium notatum*	21.7
K3 on Richard's solution	24.1
Autoclaved kernels inoculated with K3	22.6
Living kernels inoculated with K3	19.9

To compare the filtrates of *Penicillium oxalicum* with those of *P. notatum* and K3, all 3 fungi were grown on Richard's solution, on autoclaved kernels, and on living kernels, 14 days. Wounded, surface-sterilized kernels were then grown in the filtrates, sterilized by filtration, from these sources. From table 7 it is seen that *P. oxalicum*, an injurious species, produced a toxic filtrate, while the other 2 species, noninjurious to wounded kernels, did not.

To determine whether the time of inoculation of the kernels would affect the severity of the disease, 8 lots of kernels were placed on wet soil under bell jars covered with dark cloth; and, at 24-hour intervals, one series was wounded, inoculated, and covered lightly with soil. By the time the last series was inoculated, the plumules were beginning to break through the coleoptiles.

Table 8 shows that the time of inoculation was of significance: the earlier

TABLE 8.—*Effect of time of inoculation on the severity of the Penicillium seedling blight of corn. All kernels placed on wet soil to germinate at the same time. Inoculation at 24-hour intervals*

Kernel	Time of inoculation	Average height in centimeters
Sound Noninoculated		34.1
Wounded Inoculated	At planting	2.8
Wounded Inoculated	24 hours after planting	2.5
Wounded Inoculated	2 days after planting	3.7
Wounded Inoculated	3 days after planting	5.4
Wounded Inoculated	4 days after planting	9.7
Wounded Inoculated	5 days after planting	10.5
Wounded Inoculated	6 days after planting	15.6

the inoculation the more severe the disease; but, even 6 days after planting, inoculation caused a decided decrease in the height of resulting seedlings.

Because the strain of *Penicillium oxalicum* used in the present studies was not destructive to sound inoculated kernels, but damaged wounded inoculated kernels severely, a study was made to determine the effect of place of inoculation. Hurd (7) has reported that *P.* spp. caused more severe injury to wheat when the pericarp over the endosperm was wounded than when that over the embryo was damaged.

In the present experiment 100 kernels were wounded at the crown of the endosperm, and 100 were wounded to the same degree at the side of the scutellum of the embryo. Both lots were inoculated and planted in sterile soil.

Sixteen days later it was evident that the injury and inoculation in the starchy crown of the endosperm caused a greater stunting of the seedlings than did injury and inoculation over the scutellum. The average height of the seedlings from kernels wounded at the side of the scutellum was 10.1 cm. as compared with 2.6 cm. of seedlings from kernels wounded at the crown of the endosperm, and 21.8 cm. of those from sound noninoculated kernels. This suggests that the fungus grew on the starchy endosperm more readily than on the living cells of the embryo.

DISCUSSION

Although the importance of corn seedling injury by *Penicillium oxalicum* is not known, the organism is present in the soil and can be isolated from damaged ears in the field. Also, observation proves that seed kernels often are injured, cracked, or chipped at the crown; actual tests indicate that an average of 10 per cent of the kernels used as seed are so damaged; such damaged kernels were severely injured when inoculated with a spore suspension of *Penicillium oxalicum.*

The writer has observed *Penicillium* on the kernels of young seedlings pulled in the field; but, usually, attempts to isolate *Penicillium oxalicum* have not given conclusive proof of its presence, because of difficulty in obtaining pure cultures and in determining species.

From the experiments here reported it appears that soil moisture is a factor in determining the degree of injury suffered by corn seedlings grown from kernels attacked by *Penicillium oxalicum* through injured "seed" coats. Soil moisture, however, is apparently instrumental only when the kernels are pressed into contact with the wet soil. When the wounded and inoculated part of a kernel is in close contact with packed, very wet soil the degree of injury by the fungus is reduced. Perhaps oxygen deficiency may be the cause of poor growth of the fungus on kernels pressed into wet soil, with subsequent lessening of fungus activity and disease injury.

The results of this study are in agreement with those of Broadfoot (2) who concluded that the growth reaction of two fungi on culture media is not a reliable indication of their reaction or interaction on a host in the soil. Although there was some protection offered by *Penicillium* species and *P. notatum* against *P. oxalicum* under some conditions in the soil, antagonism was not apparent in culture. Even in the soil the protection was more marked if *P. notatum* or *P.* spp. were given the advantage of a heavy spore load or an initial start; when the soil was heavily inoculated with *P. oxalicum* there was not much protection if the kernels were inoculated with a spore suspension of *P. notatum* or *P.* spp. and planted at once.

It would seem that the protection is not a case of actual antagonism caused by toxic substances; it may be that the fungus first invading the kernel had the advantage of occupation and slowed up subsequent infections. If *Penicillium oxalicum* invaded first it caused severe injury to the seedling; if *P. notatum* or K3 invaded first there was less injury, for these fungi were themselves not harmful, and, by occupying the territory, inhibited invasion by *P. oxalicum,* just as a heavy stand of bluegrass will tend to keep out crabgrass.

When a wound is present in the pericarp over the endosperm *Penicillium oxalicum* grows rapidly after inoculation. The fungus is probably chiefly saprophytic, as suggested by Johann (9), living on the available carbohydrate in the endosperm, but it produces a toxic substance that kills the embryo. Even though the fungus eventually enters the seedling, principal injury appears to be caused by some toxic substance produced by it, which the embryo absorbs from the endosperm.

SUMMARY

Penicillium oxalicum caused a severe stunting of corn seedlings grown from kernels with damaged "seed" coats when the kernels were inoculated with a spore suspension or were planted in soil inoculated with the organism.

When wounded and inoculated kernels were pressed into contact with very wet soil the degree of injury caused by the fungus was reduced.

Penicillium sp. (Isolate K3), isolated from a diseased ear in the field, and *P. notatum* offered wounded kernels some protection against infection by *P. oxalicum* from the soil. No antagonism could be observed between these species and *P. oxalicum* in culture.

The protection was increased when the protecting fungus was given a time advantage, and when the kernel was very heavily inoculated with the protecting fungus.

Penicillium notatum and K3 caused no damage to wounded and inoculated kernels planted in sterile soil in the greenhouse.

The sooner after germination the wounded kernels were inoculated with *Penicillium oxalicum*, the greater was the degree of injury by the fungus.

A toxic substance was produced by *Penicillium oxalicum* on Richard's solution, on autoclaved kernels, and on living kernels. *P. notatum* and K3 did not produce such a toxic substance.

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THE EFFECT OF FLUE-CURING ON THE INFECTIVITY OF ORDINARY TOBACCO MOSAIC VIRUS (TOBACCO VIRUS 1)[1]

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INTRODUCTION

The field control of ordinary tobacco mosaic depends upon a thorough knowledge of the sources of inoculum. The known factors involved in the control of the disease have been reviewed by Wolf (7), James Johnson (2), and Valleau and E. M. Johnson (6), for flue-cured, Burley, and other air-cured types of tobacco. In these reports attention was directed to the probability of the virus remaining infectious throughout the flue-curing process. If this be true, the use of flue-cured tobacco, by growers, before it is manufactured into cigarettes and other commercial forms of tobacco may possibly account for a certain amount of field infection in the flue-cured districts.

Preliminary studies, dealing specifically with the ability of the virus to retain its infectiousness in flue-cured tobaccos, are reported by Wolf (7), James Johnson (2), and Pinckard (3). The results of these studies clearly indicated that flue-curing did not completely destroy the virus, although no data were presented to show the curing-barn conditions under which the virus was or was not destroyed. By means of a constant-temperature oven, James Johnson (2) studied the influence of temperatures on the inactivation of the virus. Exposures of from 1 to 4 hours at temperatures of 178° and 183° F. resulted in some decrease in the average number of local lesions of the virus on a hybrid test plant. As the period of exposure increased to 48 hours, complete inactivation was finally obtained at 183° F. In practice, however, the higher temperatures of flue curing vary widely, but for only short periods of time. It seemed desirable, therefore, to secure further information on the persistence of the virus under actual flue-curing conditions.

The purpose of this paper is to present the results of more detailed studies on the effect of flue curing on the infectivity of the virus in relation to the curing-barn temperatures and time required to bring tobacco leaves to the "cured" state.

METHODS AND MATERIALS

Following a severe epidemic of tobacco mosaic in the field plots of the Experiment Station at Chatham, Virginia, in 1936, diseased leaves were collected and cured in the usual manner. Inoculations of potted tobacco seedlings, with aqueous suspensions from these leaves showed that the curing process, as used in 1936, did not destroy the virus.

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For the purposes of this investigation, the usual curing process was essentially the same as that described by Garner (1). Two types of curing barns were employed. One, of concrete block construction, having a single retort, or furnace, with a flue conducting the heat across the center of the floor, around the sides, and with exit over the retort. In the other barn, which was of ordinary frame construction, the heating plan was almost identical with that described by Garner. Pine logs were used for fuel. The latter type of curing barn is in general use in the flue-cured tobacco sections at the present time.

Suitable samples of mosaic leaves were collected from the field and 1 disc from each of 5 leaves was cut with a No. 15 cork borer. The 5 discs were enclosed in an envelope and placed in the barn, for curing, at a central point in the lower tier. One envelope, with its discs, was removed from the curing barn after remaining for the required length of time, and saved for future inoculation tests. Since the above samples were not handled after curing, except to be macerated in sterilized mortars before being used as inoculum, accidental contaminations were believed to have been minimized. Sterile cotton swabs were used to apply the inoculum and sterile pot labels were used to hold the leaves while inoculating potted seedlings of tobacco grown in the greenhouse. Three tobacco plants were inoculated with each sample of material tested.

Air and leaf temperatures, developing within the barns during the curing process, were registered either by means of a thermograph, mercury thermometers, or by a thermocouple method, using a student's potentiometer and copper constantan junctions. The reference junctions were held at the temperature of melting ice, while the hot junctions were embedded in the midribs of leaves or wrapped with cotton and suspended at various positions within the barns. All thermometric equipment was calibrated against the temperatures of melting ice and boiling distilled water. Readings to the nearest whole degree Fahrenheit were considered to be sufficiently accurate for the purposes of this study.

Loss in weight of duplicate leaf samples, consisting of 3 entire leaves from both healthy and diseased plants, was measured by enclosing the samples in cheese-cloth bags suspended from the central lower tier. At frequent intervals the samples were removed from the barns for weighing, which was done as rapidly as possible after cooling. Desiccators were not used since the combined weight of the entire leaves in each sample did not seem to warrant the added precaution.

The data were taken in the various curing barns in the sequence of filling, which was also the sequence of harvest, *i.e.*, the lower leaves from the plants were harvested first and as maturity progressed the leaves from the upper portion of the plants were harvested last. In general, slightly more heat was applied to later curings than was required for the earlier curings.

A commercial type of oil-burning tobacco-barn heater was installed in one of the barns in 1938. Continuous temperature records were kept on 5 curings in this barn to determine if the increased efficiency in curing-barn heating was above or below the thermal limit of the virus in flue-cured tobaccos.

RESULTS

Repeated inoculations with *tobacco virus 1,* obtained from the previous year's flue-cured crop gave consistent infection on potted seedlings in the greenhouse. Inoculations of seedlings in field plots resulted in the usual disease development and in losses (4) comparable to those reported by Wolf and Moss (8). Experiments in Virginia indicated that, even though the virus may have been partly inactivated by the heat of flue curing, its infectiousness upon inoculation of field-grown plants was seemingly no different than inoculum from other sources.

To determine if the virus commonly passed through the curing barns, under actual conditions of curing, samples of diseased leaves were studied from the first 5 barns cured in 1937. The results of these experiments, summarized in table 1, showed that diseased leaf samples removed from the lower, middle, and upper tiers of the first 4 barns and samples from the lower and middle tiers of the 5th barn retained their infectiousness. The leaf samples from the upper tier of barn No. 5 were disease-free.

Detailed information was then sought on (a) the time and temperature at which complete virus inactivation took place at a central point within the barns, (b) comparative temperatures at other points within the barns, and (c), the loss in weight of leaves as an indication of when curing was complete. Progressive records were kept on time, temperature, loss in weight of leaves, and the presence or absence of the virus in leaf samples. Although over 1000 temperature readings were taken and over 600 samples of leaves were tested for the presence or absence of the virus, only small portions of the data are presented. For the sake of brevity, these data are summarized in table 1.

The time and temperatures at which complete inactivation took place within any barn could only be approximated. As figure 1 indicates, temperature changes in wood-fire barns are difficult to control and changes may be rapid. Weather conditions at the critical temperature of complete virus inactivation in actual flue-curing practice were found to have an important bearing on the stability of curing-barn temperatures. Under these conditions, as the data in table 1 indicate, complete inactivation of the virus took place at temperatures of not less than 180° F.

Substantial temperature differences were found at different places within each barn studied. The greatest differences were found at the higher temperatures encountered. In an extreme case a maximum barn temperature of 256° F. was found necessary in order to bring the coolest portion of the barn up to a maximum of 196° F. It should be pointed out that the temperatures in practically all tobacco curing-barns are regulated by a thermometer suspended from the lower central tier and that the temperatures at this point are considered to be average for the barn. A study of figures 1 and 2 show the temperature range at other points within wood-fired barns. A 50-degree difference in peak temperatures between the coolest and warmest portions of a barn was not uncommon.

TABLE 1.—*Summary of the maximum and minimum curing-barn temperatures, and the elapsed curing-barn time in relation to the loss in weight and the persistence of tobacco virus 1 in ripe tobacco leaves. Chatham, Va., 1937*

Curing-barn	Elapsed curing-time	Max. and min. air temperatures at lower tier	Plants inoculated	Leaf samples[a] collected	Presence or absence of the virus in samples[b]	Loss in wt.[c]	
						Healthy	Diseased
No.	*Hrs.–Min.*	°*F.*	*No.*	*No.*		*Per cent*	*Per cent*
1	0–0	76	18	6	+	....	....
	88–30	191	18	6	+	....	....
	95–30	136	18	6	+	....	....
2	0–0	93	18	6	+	....	....
	97–45	188	18	6	+	....	....
3	0–0	93	18	6	+	....	....
	95–30	196	18	6	+	... ...	....
4	0–0	93	18	6	+	. . ....	.. ....
	[illegible]	196	18	6	+	....	....
5	0–0	73	18	6	+	....	....
	88–30	193	18	6	+	. ....	....
	88–30	193	18	6	0[d]	. .....	....
6	0–0	86	6	2	+	0	0
	73–0	158	6	2	+	88.1	88.2
	84–30	198	6	2	+	88.5	88.5
	91–30	178	6	2	0	88.5	88.5
7	0–0	73	6	2	+	0	0
	80–0	175	6	2	+	88.5	89.0
	84–0	193				88.5	89.0
	87–0	180	6	2	0	88.5	89.0
8	0–0	73	6	2	+	0	0
	87–0	200	6	2	+	86.8	84.6
	88–0	200				86.8	84.6
	96–0	134	6	2	0	86.8	84.6
9	0–0	73	6	2	+	0	0
	111–30	130	6	2	+	85.8	85.5
	112–30	203			..	86.1	85.5
	114–0	208	6	2	0	86.1	85.5
10	0–0	78	6	2	+	0	0
	86–0	128	6	2	+	80.2	74.5
	89–0	178	6	2	+	..	... ..
	90–30	208	6	2	0		....
	93–45	198	6	2	0	83.8	83.5

[a] Five leaves used per sample, as explained in text.

[b] Results indicated as follows: +, virus present in leaf samples; 0, virus not found in leaf samples.

[c] Three entire leaves per sample, in duplicate, as explained in text.

[d] Refers to samples collected from the upper tier.

TABLE 1—*Continued*

Curing-barn	Elapsed curing-time	Max. and min. air temperatures at lower tier	Plants inoculated	Leaf samples[a] collected	Presence or absence of the virus in samples[b]	Loss in wt.[c]	
						Healthy	Diseased
No.	*Hrs.-Min.*	*°F.*	*No.*	*No.*		*Per cent*	*Per cent*
11	0–0	75	6	2	+	0	0
	69–45	132	6	2	+	75.7	75.3
	83–45	168	6	2	+		
	86–0	198	6	2	0	84.5	85.2
12	0–0	73	6	2	+	0	0
	84–0	183	6	2	+	83.5	83.8
	86–10	198	6	2	0	83.5	83.8

[a] Five leaves used per sample, as explained in text.

[b] Results indicated as follows: +, virus present in leaf samples; 0, virus not found in leaf samples.

[c] Three entire leaves per sample, in duplicate, as explained in text.

Differences in loss of weight between healthy and mosaic leaves were found to be insignificant. As the harvest progressed, the total dry matter in all leaves increased from an average of 12.2 per cent in curing barn No. 6 to 16.2 per cent in curing barn No. 12, even though final drying temperatures were generally several degrees higher in the later curings.

The temperatures at which leaves were cured and dry apparently varied with the relative humidity of the outside atmosphere. In barn No. 6, the leaves were cured and ready for cooling after a temperature of 158° F. was reached 73 hours after the start. Similarly, the leaves in barn No. 7, were cured at a temperature of 175° F., 80 hours after the start. In barn No. 9, the leaves were almost dry at a temperature of 130° F., 111 hours after the start. In this instance higher temperatures were not secured for several hours because of cold winds. Adequate data on drying were not obtained in barns 10 and 11, but the leaves in barn No. 12 were cured and dry at a temperature of 183° F., 84 hours after the start.

Maximum curing-barn temperatures obtained with a regulated oil-burning heating apparatus in 1938, ranged from 167° to 175° F. At no time during any curing was the temperature above 150° F. for more than 15 hours. Unfortunately, samples of mosaic leaves were not collected from these 5 barns.

DISCUSSION

The work of James Johnson (2) on the influence of higher temperatures over long periods of exposure, as in flue curing, on the inactivation of *tobacco virus 1* showed that the virus was not completely inactivated at a temperature of 178° F., or lower, when exposed from 24 to 48 hours. Neither was the virus completely destroyed at 183° F. for 12 to 24 hours, although it was destroyed at this temperature when held for longer periods of time. The results of Price (5), and others, on the thermal death time of the virus in

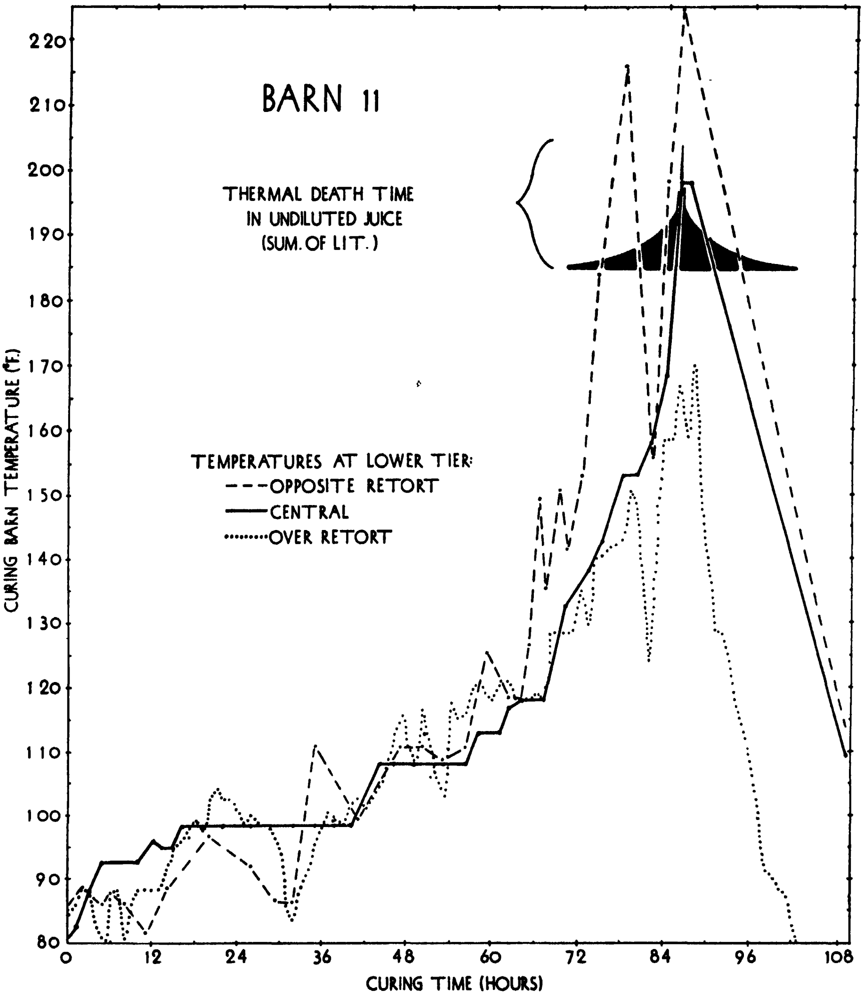

FIG. 1. Graphic illustration of temperature variation at several points within flue-curing barn No. 11, of concrete block construction, Chatham, Va., September, 1937.

undiluted juice is roughly similar and is graphically presented in figures 1 and 2, for comparison with curing-barn temperatures.

With the thermal death time of the virus approximately established at temperatures above 180° F. for periods of not less than 24 hours and with optimum curing-barn temperatures ranging below 180° F. for final drying periods of not more than 12 to 16 hours, it appears, therefore, that the flue-curing process, is of doubtful value in destroying the virus.

Tobacco mosaic always has been a serious disease in most of the flue-cured districts of the Southeastern States. Although the disease is being successfully controlled in the flue-cured areas in experimental plantings, many intricate problems associated with successful control on the farm are yet to be investigated. Owing to the differences of climate, soil characters, tobacco

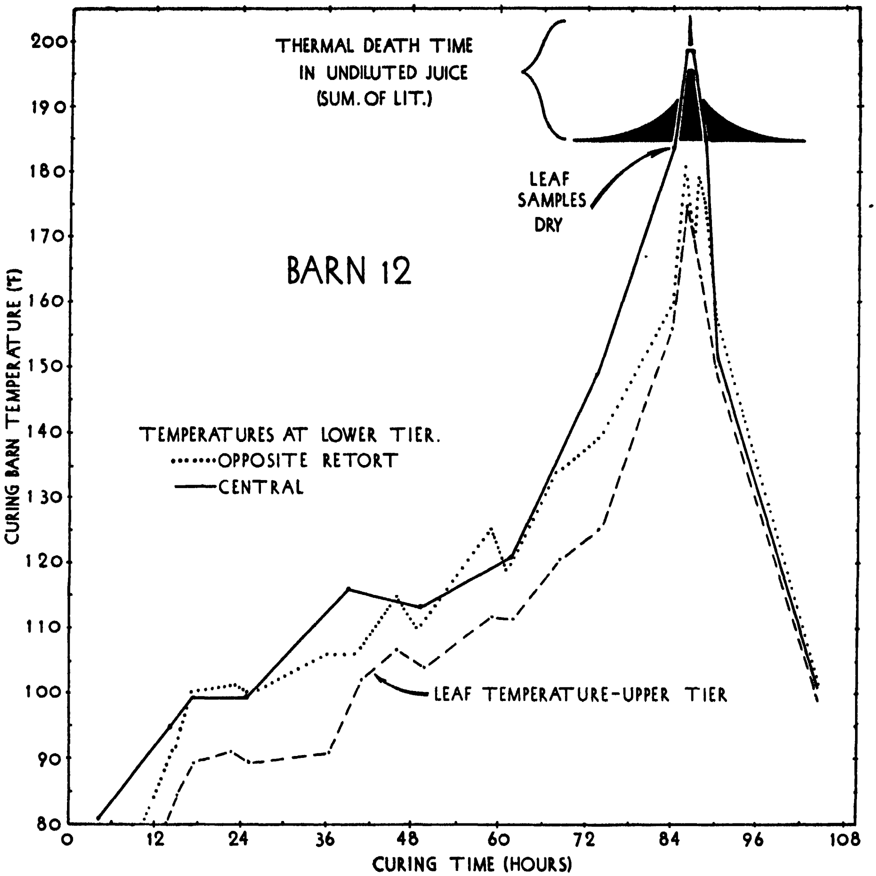

FIG. 2. Graphic illustration of temperature variation at several points within flue-curing barn No. 12, of wood construction, Chatham, Va., September, 1937.

types, and culture, the importance of overwintering and sources of primary infection is largely one of degree with respect to the cultural practices within an area. Even within the Old Belt of the flue-cured type, two distinct cultural practices exist in the production of flue-cured tobacco. The most important is the "priming" method of harvest in which the leaves are removed and cured at final drying temperatures of 170° to 180° F. The other, and older practice is one in which the stalk is cut and the entire aboveground portion of the plant is cured at temperatures of 220 to 240° F. These latter temperatures, if uniformly maintained throughout the curing barns, for the usual time, are apparently adequate to destroy the virus of tobacco mosaic. Since "stalk cut" tobacco also is used in the manufacture of cigarettes, and since it was the principal "type" grown in Virginia up to within a few years ago, growers and investigators alike were essentially correct in assuming that the temperatures of flue curing were sufficient to destroy the virus.

Following the change from "stalk-cutting" to "priming," less heat was required. With the advent of oil-burning heating units, and for economic reasons, curing-barn temperatures are being lowered and more importance is being attached to the relative humidity than to the temperatures of final drying. The data from several of the curing-barns used in this study clearly indicate that efficient curing is not entirely dependent upon temperature but upon the conditions that make for satisfactory drying of the leaf. These conditions may be satisfied on a dry day at comparatively low temperatures, far below the thermal limit of the virus. During such weather considerable quantities of the virus-infected leaves may be expected to retain their infectiousness upon curing. Cool, windy weather conditions during the flue-curing season invariably result in extensive temperature variations within barns, so that portions of the "cure" may not be thoroughly dried or heated sufficiently to completely inactivate the virus.

With the present type of curing barns, it appears to be neither desirable nor practical to attempt to maintain uniform temperatures lethal to the virus. Complete destruction of the virus throughout a barn of flue-cured tobacco appears to be an accidental accomplishment except in the case of "stalk-cut" tobaccos. As Wolf (7) has pointed out, most fields of flue-cured tobacco are from 50 to 100 per cent mosaic-affected at the close of harvest, although symptoms are not always apparent in the harvested leaf. In view of these conditions, and because of only incomplete destruction of the virus in flue-curing, it seems wise to consider flue-cured tobaccos as a potent reservoir of *tobacco virus 1.*

A critical study of flue-curing, together with a study of the precise thermal death time of the virus in flue-cured leaves, might possibly result in a system of curing-barn management that would lead to the complete destruction of the virus in flue-cured tobaccos. The perfection of such a procedure would undoubtedly assist in reducing the amount of virus carried over winter.

SUMMARY

Flue-cured tobaccos have been shown to be carriers of infectious *tobacco virus 1.*

The virus remained infective in flue-cured leaves throughout 4 of the first 5 barns cured and in the lower and middle tiers of the fifth barn.

Although the virus was completely inactivated in the lower tiers of 7 barns of cured tobacco in 1937, the final drying temperatures at this position were substantially higher than those ordinarily required for curing. In other portions of the same barns, maximum temperatures were both above and below the thermal limit of the virus.

Wide temperature differences, amounting to as much as 50° F., were found within the same wood-fired barn.

A study of the maximum temperatures attained in 5 barns of tobacco cured with an oil-burning heating unit showed that at no time was the thermal death point of the virus reached.

Leaves infected with *tobacco virus 1* were found to lose weight, cure, and become dry at the same temperature and time as did noninfected leaves.

The temperatures required for a satisfactory "cure" varied, but were found to be substantially below the thermal death temperature and time for complete virus inactivation.

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PURIFICATION OF NICOTIANA VIRUS 6 PROTEIN[1]

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Nicotiana virus 6 (8), which causes the mild-dark-green mosaic of tobacco (5), differs from *Nicotiana virus 1,* which causes the common mosaic of tobacco, in that it does not increase in tomato (5), does not induce local necrotic lesions or systemic infection on beans, *Phaseolus vulgaris* L. var. Scotia (7), induces local necrotic lesions on *Nicotiana sylvestris* L. (7), and reduces the plastid pigments to a less extent than does *Nicotiana virus 1* in certain varieties of tobacco (7). The virus, like *Nicotiana virus 1,* induces local necrotic lesions on *N. glutinosa* L. and *N. langsdorfii* L. (7). The virus was collected by the junior author on *N. glauca* L. growing in the Canary Islands (5).

The purpose of this paper is to report the results and observations obtained in studies on the crystallization[3] of *Nicotiana virus 6,* and to report certain differences between the "crystals" of this virus and those of *Nicotiana virus 1.*

METHODS AND MATERIALS

Source of the infectious plant juice used in all but one test was *Nicotiana tabacum* L., variety Wisconsin Havana seed, grown under greenhouse conditions until the plants were producing flower buds. Leaves from the plants were frozen, macerated while frozen through a food chopper, and kept frozen until needed. One test was carried out with a virus extract from young tobacco plants.

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[1] Studies conducted under Bankhead-Jones Project S.R.F. 2-17, U. S. Department of Agriculture, Bureaus of Plant Industry and Chemistry and Soils cooperating.

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[3] The word crystal and its forms are used for the sake of brevity to indicate particles that may not be true crystals but meso- or para-crystals or condensation products.

Crystallization of the virus protein was obtained by a modification (16) of Stanley's improved method (10) for the isolation of *Nicotiana virus 1* protein. The frozen material was heated to 40° C. in presence of disodium phosphate (35.8 g. per 1000 g. of frozen material), and the liquid pressed through doubled cheesecloth by wringing with the hands. This solution, while warm, was filtered by suction through a celite cake (25 g. of celite per 1000 ml. of liquid) on a Buchner funnel. The pulp from the first extraction was reextracted in an equal volume of 0.1 molar disodium phosphate solution at 40° C. This and the first extract were treated similarly.

First crystallization was obtained from the celite filtrate by adjusting the H-ion concentration to pH 4.5 with sulphuric or acetic acid (about 3N) and adding ammonium sulphate salt (175 g. per 1000 ml. of liquid) to 0.3 saturation. The liquid upon agitation possessed a velvety appearance (sheen) characteristic of suspended crystalline protein. It was allowed to stand over night at about 5° C. for further crystallization. The crystalline and other precipitated materials were separated from the dark brown liquid by filtration with suction through celite and by washing the cake with a small volume of 0.3 saturated ammonium sulphate solution at pH 4.5. The celite and adhering materials were then suspended in distilled water (about ¼ of the original volume) and the reaction adjusted to pH 8.0 with 0.5 molar disodium phosphate solution. After thorough agitation, the suspension was heated to 40° C. and the celite removed by filtration with suction through filter paper. The celite was washed 3 times with 0.01 molar phosphate buffer solution (each time with 1/12 the original volume) at pH 8.0 and 40° C. After combining the filtrates (about ½ the original volume), samples were taken for assay of infectivity and of protein nitrogen. The material from the second extraction was crystallized in a similar manner and then combined with that from the first extraction.

Recrystallization and taking of samples were carried out as in the first crystallization. Upon separating the protein from the liquid in the second crystallization, acetone (25 per cent by volume) was added to the suspension 30 minutes before filtration. This concentration of acetone is sufficient to remove coloring material from the protein solution without any apparent reduction of infectivity.

Infectivity of certain samples was assayed by the local-lesion method on *Nicotiana sylvestris*. Inoculum consisted of virus protein solutions containing 10^{-2} mgm. of protein per 1 ml. of 0.1 molar phosphate buffer at pH 7.0. The protein content of the samples was determined by micro-Kjeldahl analysis for nitrogen in the protein precipitated with 5 per cent trichloroacetic acid. This nitrogen value times 6 gives the protein content, assuming this virus protein to contain 16.55 per cent nitrogen as that reported (9) for *Nicotiana virus 1.*

The virus protein was analyzed for nitrogen by the micro-Kjeldahl method, for phosphorus by the wet-combustion method (6), for sulphur by the pressure-tube method (6) and for solids by the constant-weight at 100° C. method. Before analysis the virus protein was crystallized 5 times by means of hydrochloric acid and sodium acetate buffers and sodium sulphate salt. These conditions were substituted for those of the regular procedure to avoid the introduction of nitrogen and phosphorus into the protein from the reagents used. Sulphur could not be substituted because neutral soluble sulphate salts were necessary for crystallization. The protein, after separation from the liquid in the 5th crystallization, was dissolved from the celite filter cake and a portion of it dialized under toluol for 10 days against 0.001 molar sodium acetate at pH 7.5 and for 2 days against distilled water. Both dialized and non-dialized proteins were analyzed. Studies on the microscopic appearance and the effect of Fairchild's trypsin on the virus preparation were carried out.

RESULTS AND OBSERVATIONS

Results from the protein and infectivity assays of virus samples at various stages of purification are given in table 1. The yield of protein based on the

TABLE 1. *Data on the nitrogen content and infectivity of protein from plants infected with Nicotiana Virus 6 and from healthy plants*

Materials	Trypsin[a]	Volume	Protein		Infectivity[b]
			N./ml.	Total	
	(hr.)	*(ml.)*	*(mgm.)*	*(mgm.)*	
Virus protein:—					
Original extract		406	0.2212	538.84	8.65
2nd do		490	0.1271	373.67	
Total				912.51	
1st. crystallization original ext.		200	0.3110	373.20	3.37
do do 2nd do		200	0.0801	96.12	
Total				469.32	
2nd do		200	0.2903	348.36	4.12
3rd do		200	0.2474	296.88	5.67
3rd do without trypsin	0	50	0.2765	82.95	
3rd do do do	1	50	0.2765	82.95	
3rd do with do	0	50	0.3630	108.90	
3rd do do do	1	50	0.3511	105.33	2.16[c]
5th do do do	0	50	0.9697	290.91	
5th do do do	4	50	0.9066	271.98	
5th do do do	24	50	0.8714	261.42	
3rd do from young plants with trypsin	0	50	0.2868	86.04	
3rd do do	1	50	0.2684	80.52	
3rd do do	2	50	0.2486	74.58	
Normal protein:—					
3rd precipitation from young plants, with trypsin	0	50	0.0655	19.65	
do do	1	50	0.0294	8.82	

[a] Digestion with 1 mgm. of trypsin per 1 ml. of solution at pH 7.5 and 35° C.

[b] Average number of lesions per leaf on 40 leaves on 8 plants of *Nicotiana sylvestris* inoculated with solutions containing 1×10^{-3} mgm. protein per 1 ml.

[c] Average number of lesions per leaf on 25 leaves on 5 plants of *Nicotiana sylvestris* inoculated with solutions containing 1×10^{-3} mgm. protein per 1 ml.

celite filtrate of the combined extracts was 51.4, 38.1, and 32.5 per cent in the first, second, and third crystallizations, respectively. Separately, in the first crystallization, 69.2 per cent of the protein in the original extract and 25.7 per cent of that in the second extract were fractionated with the crystalline material. Infectivity on the basis of protein content was not appreciably changed by treatments for crystallization nor by the action of trypsin.

The results of the partial elemental analyses of one virus protein preparation are given in table 2. Analysis of other preparations and complete elemental analysis were impossible due to termination of work. It will be seen that the nondialized protein contained 11.90 per cent nitrogen, while the dialized material contained 13.28 per cent by dry weight. Whether salts in the nondialized protein solution increasing the dry weight or whether error in analysis caused this value to differ from that of *Nicotiana Virus 1* (9) is not known. Phosphorus (0.33 per cent) was obtained in the nondialized material, but 3 tests failed to detect any in the dialized protein. Sulphur having been added to the solution to obtain crystallization would be expected in the nondialized material. That measured in the dialized protein (0.16 per cent) may represent residual sulphur occluded to the protein, or protein sulphur, or both. Solids in the nondialized and dialized solutions was 1.55 and 1.21 per cent, respectively.

TABLE 2.—*Elemental analysis of Nicotiana Virus 6 protein*

Virus protein	Percentage			
	Solids[a]	Nitrogen	Phosphorus	Sulphur
Nondialized	1.55	11.90	0.33	1.33
Dialized	1.21	13.28	0.00	0.16

[a] Solids determined from the protein solution by weight.

A virus preparation containing 2.17 mgm. of protein per ml. and which had been crystallized 3 times was digested with 1 mgm. of Fairchild's commercial trypsin per 1 ml. of protein solution at 35° C. for 1 hour at pH 7.5. Nitrogen analysis (Table 1) indicated a loss of 3.28 per cent of protein due to trypsin digestion. Virus protein in solution (1.65 mgm. protein per 1 ml.) exposed to similar conditions but without trypsin lost no protein during the treatment.

Virus protein from very young infected plants was studied to determine whether materials in plants of different ages influence crystallization and whether normal protein might fractionate with the virus protein. The particles developed in repeated crystallization, as was the case in preparations from older plants. After trypsin digestion of this material in solution (1.7 mgm. protein per 1 ml.) the particulate form developed upon recrystallization at pH 4.5 and on changing the reaction to pH 3.5, whereas preparations from older plants developed the thread or fiber forms at pH 3.5. From the amount of digestion in 1 and 2 hours (6.41 and 13.31 per cent, respectively), it appears that trypsin digestion did not remove all the contaminating protein that may have inhibited the formation of fiber particles at pH 3.5. Protein solution (0.39 mgm. protein per 1 ml.) from young healthy plants under similar conditions with and without trypsin precipitated in amorphous form without any evidence of crystals. Trypsin digestion of this material for 1 hour removed 55.11 per cent of the protein. Whether this normal protein is capable of crystallizing in the presence of virus protein or is occluded to the crystalline virus protein is not known. In these preparations a digestible protein was associated with the nondigestible virus protein.

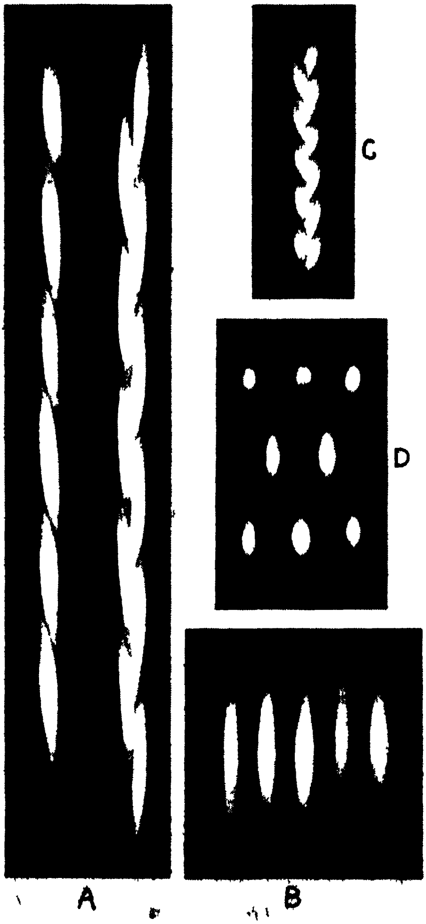

FIG. 1. "Crystals" of virus proteins. A and B. *Nicotiana Virus 1.* A. Single chain and two parallel chains of crystals; B, single crystals. C and D. *Nicotiana Virus 6.* C. Two parallel chains of crystals; D, separated crystals. Drawn by H. H. McKinney from magnification × 5000 with dark-field illumination.

The purified virus protein was studied microscopically by direct and dark-field illumination over a range of hydrogen-ion concentrations from pH 7.5 to 3.5 at 0.5 intervals. Observations with direct illumination failed to reveal any particles in the preparations adjusted to pH 7.5, 7.0, 6.5, and 6.0. At pH 5.5, however, many small particles or crystals and thread-or-fiber-like particles were observed. These increased in number and size with the change of reaction to pH 4.5. Further change in reaction had no perceptible effect on the size or number of particles. The particles were hyaline and possesed much Brownian movement.

With dark-field illumination the preparation at pH 7.5 was the only one free of refractive particles. At pH 7.0 a few extremely small or slightly refractive particles were visible. At pH 6.5 the particles were larger or more refractive and appeared to be elongated. At pH 6.0 a few of the particles reached about the maximal size and they were elliptical in shape, but many small indistinct particles were also present. Further changes in reaction to pH 5.5, 5.0, and 4.5 did not noticeably change the size or refractive characteristics of the largest particles, but did cause more of the smaller particles to grow to the maximal size. From pH 4.0 to 3.5 there was no apparent change in the number or the size of the particles.

The most distinct particles in each preparation were measured and the size ranges are given in table 3. Typical particles observed in the dark-field are illustrated in figure 1. Comparisons are made with the "crystals" of *Nicotiana Virus 1* at pH 4.5. The microscopic particles of *Nicotiana Virus 6* and *Nicotiana Virus 1,* which were measured and illustrated, represent the microscopic particulate units of these virus proteins under the conditions studied.

TABLE 3.—*Sizes of virus-protein particles in preparations adjusted to different H-ion concentrations. Measurements made at magnification of 1500 × with dark-field illumination. Distinct particles only were measured*

Virus preparations	H-ion concentrations	Dimensions of particles in microns	
		Width	Length
	(pH)		
Nicotiana Virus 6,			
tryptic digestion	7.5	.4–.5	.8–1.0
no tryptic digestion	7.5	no particles	no particles
do	7.0	.3–.4	.3– .4
do	6.5	.4–.5	1.0–1.3
do	6.0	do	1.0–1.6
do	5.5	do	do
do	5.0	do	do
do	4.5	do	do
do	4.0	do	do
Nicotiana Virus 1,			
no tryptic digestion	4.5	do	3.2–4.2

At pH 6.5 a few of the particles were aggregated in pairs end-to-end. As the pH value was reduced the particles aggregated in chains, the adjacent particles overlapping slightly at the tips. At pH 4.0 from 8 to 10 or more particles made up these chains and the number of chains was very great. These chains when in undisturbed mass tend to become oriented with their long axes parallel. Very frequently two chains appear as miniature spikes of barley or wheat, as illustrated in figure 1. With transmitted light the particles making up the chains are not properly resolved; thus the very short chains—2 or 3 particles—appear as long crystal-like particles and the very long chains resemble solid threads or fibers. Sometimes, many parallel chains form wide compact bands, and these may assume an undulating form.

The rate at which the virus-protein particles settle out on standing is influenced by differences in H-ion concentrations. At pH 4.0 the pure white precipitate comes down over night at 5° C. With each increase of 0.5 pH in reaction the time increases 1 to 3 days, up to pH 5.0. At pH 5.5 complete precipitation may require as long as 2 weeks. On standing 8 weeks there was no precipitate in the preparations at pH 6.0 and higher. At pH 6.0 and above there was turbidity, but this reduced with decrease in H-ion concentration.

After standing for 8 weeks at 5° C., samples were carefully removed from the top fluid from each pH preparation and examined in the dark-field microscope. Samples at pH 6.0 and 6.5 contained many pointed particles and a few very short chains of particles. Agitation did not appreciably change the picture in subsequent samples from these preparations. Only indistinct particles and a few very small refractive particles were found in samples at pH 7.0 and 7.5 The clear top fluids at the lower pH values where precipitation was complete revealed no refractive particles in the dark-field.

A portion of the preparation that was adjusted to pH 7.5 and free of any microscopic particles (5.8 mgm. protein per ml.) was digested with Fairchild's trypsin. After digestion and at pH 7.5 the preparation contained many elliptical particles, chains, and "barley-head" aggregates. The particles were very slightly smaller than those in the preparation at pH 4.0 when no trypsin was used; otherwise, the 2 preparations were essentially alike when examined by means of dark-field illumination. On changing the reaction of this preparation to pH 4.5 there was essentially no change in the microscopic particles.

Stream-double refraction (14) of the virus preparation without trypsin was conspicuous at pH 7.5, but no particles were visible by dark-field illumination. At pH 4.5, where the particles were highly refractive by dark-field illumination, the stream-double refraction phenomenon was disturbed. Apparently the precipitation of the protein in this suspension interfered with the typical stream-double refraction. According to these observations, the protein in solution at pH 7.5 without trypsin contains particles below the size visible by dark-field illumination and high magnification. Whether these particles at pH 7.5 represent the greatest degree of dispersion of the micellular units that remain infectious is not known.

DISCUSSION

Correlation of infectivity and protein content of *Nicotiana Virus 6* at various stages of purification indicates a close relationship between infectivity and protein. Purity of the protein, however, does not appear to be established by these crystallization procedures, since the crystallized protein apparently contained 2 fractions, one of which is susceptible to trypsin digestion and the other resistant. Since material crystallized from plants that had been infected for a short time contained more digestible protein than that from plants diseased for a longer period, and since normal protein was highly digestible, it appears that normal protein may be the contaminating protein in the virus material. Crystalline *Nicotiana Virus 1* under serological analysis (2, 3) also shows the presence of normal protein. Whether the digestible protein was precipitated along with the virus protein, or was occluded to it, is not known. Some normal proteins in the healthy plants were precipitated at the reaction and salt concentration favorable for crystallization of virus protein. If the two proteins possessed similar solubilities in slightly alkaline and acid reactions in presence of salts for buffers and for salting out, recrystallization would not fractionate the proteins. The action of trypsin apparently removes the contaminating protein without greatly affecting the infectivity of the virus protein. The percentage of digestible protein, however, was much less than the percentage error of the limited infectivity assay. This decrease of the digestible or normal protein with increase in the length of time that plants are infected is similar to the results reported (4, 12) for *Nicotiana Virus 1*. The normal protein may be converted directly or indirectly into virus protein, as previously suggested (4), or the diseased plants in their metabolism may change the normal protein built up prior to infection while the resistant virus protein accumulates. The decrease in normal protein with the length of time plants are infected might result also from either its break-down into products not precipitated by trichloroacetic acid, and thus not detected as protein, or from its combination and fixation to the structural elements of the plants, and thus would not be fractionated from the diseased plants in the expressed juice. Diseased plants, although systemically infected, possess localized areas that remain normal and virus-free. The areas probably continue to produce the characteristic protein formed by normal plants. As the disease progresses, the percentage area of these isolated normal areas may decrease to the extent of percentage reduction of normal protein.

The action of trypsin on the virus protein at pH 7.5 not only digests a small amount of the protein or contaminating protein, but changes the physical state of the protein. Before tryptic digestion the virus protein is in solution at a high degree of dispersion, according to the transparency of the liquid and absence of refractive particles in the dark-field. In the presence of trypsin at pH 7.5 the protein condenses into typical elliptical particles like those obtained at pH 4.5 without trypsin. This condensation at pH 7.5 suggests that the protein becomes insoluble because of the presence or action of trypsin. Whether the isoelectric point is changed or the protein is reorganized into an insoluble form at this reaction is not known. The elliptical particles, which developed after trypsin treatment, had a tendency to cohere into chains, threads, and bundles. Obviously, the action of trypsin at certain stages of purification alters particle size and filterability of the virus protein independently of the H-ion concentration. Concentrated suspensions of the particles at pH 4.5, without trypsin, aggregated into threads upon standing.

The superiority of dark-field illumination over direct transmitted light for the microscopic study was striking in the case of *Nicotiana Viruses 1* and *6*. Large particles and the apparently solid threads and fibers by transmitted light were found by means of the dark-field to be aggregates of smaller microscopic particles. The dark-field also brings into view many small particles that are not visible with transmitted light. The large microscopic particles of the two virus proteins show very decided differences in their length, those of *Nicotiana Virus 1* being much longer. Their relative width dimensions are about the same as is shown in figure 1 and table 3.

Filterability of the virus protein through celite cakes at pH 8.0 and nonfilterability and precipitation into elliptical particles at pH 4.5 suggest that the isoelectric point of this protein is near pH 4.5. Nonfilterability probably is attributable to the mechanical retention of the protein particles in the interstices of the celite filter cake. If some of the protein remained in solution at this reaction it probably was adsorbed to the celite during filtration. Upon changing the reaction to pH 8.0 the particles dissolve into highly dispersed solutions and the protein passes through the celite cake into the filtrate. At this reaction adsorbed virus protein would be eluted. Similar results should be obtained by filtration through bacteriological filters. These results favor the particle-size interpretation of nonfilterability of tobacco-mosaic virus (15) at pH 4.5 through Berkefeld "W" candles. They also suggest an explanation for the failure to obtain tobacco-mosaic virus in the filtrates through positively charged filters (15) at either pH 8.5 or 4.5. The protein at pH 8.5 (as anion) probably was adsorbed by the positively charged surface of the filters, while at pH 4.5 the protein probably was precipitated into particles that were retained mechanically by the interstices of the positively charged filter.

The relationship of degree of dispersion of the virus protein to H-ion concentration, as indicated by stream-double refraction and dark-field illumination, shows similarity to the reversibility of H-ion-induced dissociation-association reactions of other proteins, as evidenced by sedimentation analysis (13) with the ultracentrifuge. It also has bearing upon the theory (1) that a critical particle size may be regulated by H-ion concentrations to allow the optimal dispersion necessary for living matter—high dispersion of true or crystalloid solution associated with high surface and kinetic activity and thus high reaction velocities or lysis, while low dispersion with low surface and kinetic activity and, thus, low reaction velocities or aggregation. In the H-ion concentration range of pH 7.5 to 4.5, dissociation-association reactions of the protein were reversible. Acidity aggregates the protein and alkalinity dissociates it.

The virus protein prepared by these crystallizing procedures and dialized for 12 days appears to contain less nitrogen (13.28 per cent) than *Nicotiana Virus 1* protein (16.55 per cent). Since the nondialized virus protein prepared in the absence of nitrogenous reagents contained 11.90 per cent nitrogen, it appears that all the nitrogen was retained by the protein during dialysis. The presence of dializable salts in the nondialized solution and their accumulation in the dried material probably accounted for the discrepancy of nitrogen values in the two materials. Phosphorus in the nondialized material apparently was separated from the protein by dialysis at pH 7.5. Whether this phosphorus dialized from the active virus protein, as suggested for *Nicotiana Virus 1* protein (11), or whether the protein contained no phosphorus within its structure, but dializable phosphorus occluded to the protein from the infectious plant juice, is not known. Preparation of the material without the addition of any phosphorus chemicals precludes its origin from the reagents used. Phosphorus, as a part of the protein, would classify it as a nucleoprotein. It is possible that the prolonged dialysis at pH 7.5 sufficed to allow the phosphorus portion to separate from the protein and to dialize. The sulphur analysis is subject to error because of the addition of sulphate salts to the protein solutions to induce crystallization. The value obtained in the dialized material may, however, represent protein sulphur.

SUMMARY

Nicotiana virus 6 was purified from crude plant juice by crystallization at pH 4.5 and 0[illegible] saturation of ammonium sulphate.

Infectivity of the diseased plant juice was associated with a protein resistant to tryptic digestion.

Elemental analysis of the dialized protein indicated 13.28 per cent nitrogen, no phosphorus, and 0.16 per cent sulphur, while nondialized protein contained 11.90 per cent nitrogen, 0.33 per cent phosphorus and 1.33 per cent sulphur.

By dark-field illumination the unit crystals of the protein are elliptical particles, measuring 0.3 to 0.5 × 0.3 to 1.6 μ, while unit crystals of *Nicotiana Virus 1* protein are needle-like, 0.4 to 0.5 × 3.2 to 4.2 μ. Both virus proteins, on standing, aggregated into threads or bundles of unit crystals.

Nicotiana virus 6 protein was dispersed at pH 7.5 to the degree of no visible particles, and precipitated at pH 4.5 into elliptical crystals. At intermediate H-ion concentrations, the size and number of precipitated particles were directly proportional to the acidity. At pH 7.5, in the presence of trypsin, the protein was precipitated into typical crystals.

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STONY PIT, A TRANSMISSIBLE DISEASE OF PEARS[1]

J. R. KIENHOLZ[2]

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A deformity and pitting of Bosc pear fruits in Oregon was observed in commercial orchards by the writer in 1931. A roughened-bark condition resembling apple measles was often associated with the fruit pitting on diseased trees. The production of numerous "stone cells" beneath or surrounding each fruit pit, and the woody structure of the deformed fruit, has suggested the common name "stony pit" for the disease.

Many growers are of the opinion that the disease is a form of drought spot or corky core of apples. Careful irrigation and cultural practices, however, do not eliminate the trouble. Observations indicated that soil types, tree age, or the use of various fertilizers, were not correlated with the incidence of the disease. This paper constitutes a preliminary report of experiments started in 1934 to determine the cause of the pear trouble.

DISTRIBUTION AND IMPORTANCE

Stony pit is not a disease of recent origin. It is known to have been present in Oregon for many years. According to S. M. Zeller and F. C. Reimer[3] of the Oregon State College, the disease is prevalent both in the Willamette and Rogue River valleys. Mature pear trees bearing diseased fruit have been seen in the Hood River Valley. J. R. Magness of the Bureau of Plant Industry, United States Department of Agriculture, observed two

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[1] Cooperative investigation with the Oregon Agricultural Experiment Station.

[2] The writer is indebted to Dr. John W. Roberts and Dr. Lee M. Hutchins of the Division of Fruit and Vegetable Crops and Diseases for suggestions concerning the manuscript.

[3] Dr. Zeller suggested the disease might be of virus origin, and both he and Professor Reimer have experiments under way on certain phases of the trouble.

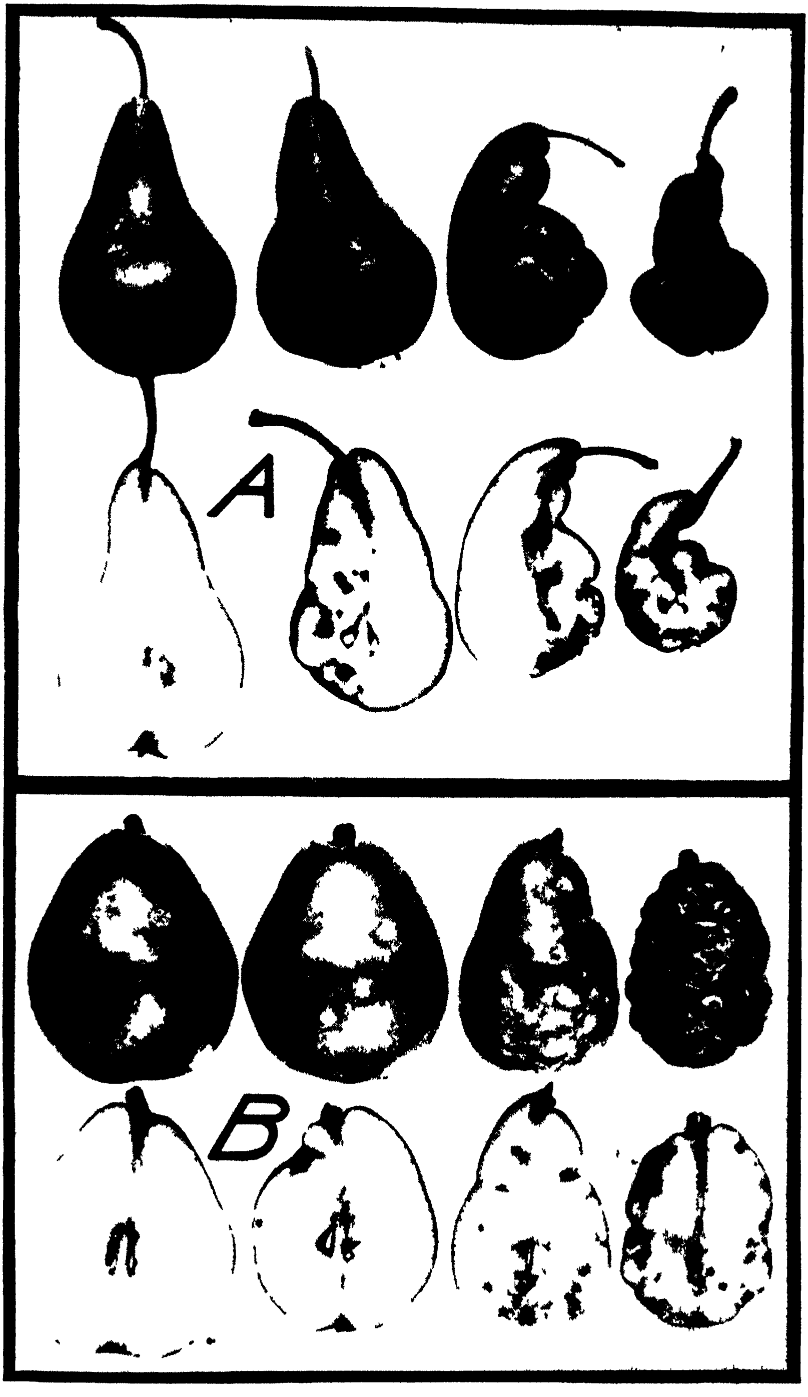

FIG. 1. Stony pit symptoms on pear fruits. Healthy fruit at left. *A.* Variety Bosc. *B.* Variety Anjou.

old Bosc trees in the San Juan district of California about 1925 that produced fruit so gnarled and pitted that it was worthless. He states[4] that the disease was apparently identical with stony pit. Various investigators who have seen stony pit in Oregon have identified it as the same disease present on Bosc pears in California and Washington. Its presence outside of the Pacific Coast States has not been ascertained.

Losses caused by the fruit pitting are severe. From 10 to 80 per cent of the Bosc *culls* at the packing houses are attributable to stony pit. Surveys made in the Hood River Valley have shown almost every Bosc orchard to contain from a few to as many as 70 per cent of the trees affected to some degree.

SYMPTOMS OF STONY PIT

On the Fruit of Bosc: Stony pit can best be identified by the symptoms on the fruit. The first indication of the trouble on Bosc pears appears in from 10 to 20 days after petal fall and consists of dark green areas just under the epidermis of the fruit. The lack of growth in these areas, and the rapid development of the surrounding tissues, results in deeply pitted or deformed fruit at maturity. The borders of the deformed areas sometimes remain dark green and suggest the halo encircling certain virus spots on other plants. The tissue at the base of these pits generally becomes necrotic or corky, and in severely infected fruits, a concentration of the corky tissue occurs near or within the "grit cell ring" (Fig. 1, *A*). The most striking feature of the diseased fruit, however, is the production of numerous sclerenchyma cells beneath or surrounding the pitted areas. Fruits bearing several pits become gnarled and so woody that they are difficult to cut with a knife.

One or all fruits on a tree may show the pitted condition. Even in cases where all the fruits on a tree show the symptoms, some may contain a single pit, while others may be severly deformed as a result of numerous diseased areas. When a single pit is present, the injury closely resembles that caused by the tarnished plant bug, *Lygus pratensis* L. The insect-induced pits lack the necrotic tissue and tend to be more regular and conical in shape, but frequently produce some stone cells below.

On the Bark of Bosc: Healthy Bosc trees generally show a natural bark cracking as they become mature (Fig. 2, *A*).

A "measled" bark condition is associated with the fruit pitting on diseased Bosc trees in the later stages. On this variety small pimples may appear on the bark of 1- or 2-year-old twigs. The cortex under the pimples becomes slightly water-soaked in appearance. Later in the season, or during the following year, the epidermis cracks and the underlying tissue collapses. The cracking and shrinking of the bark tissue occurs in a somewhat concentric manner, so that a partial target canker effect is produced on small limbs (Fig. 2, *B*). The continued cracking and growth of intervening healthy tissue cause older bark to assume a ribbed appearance. One pear grower has used the term "oak bark" to describe the bark symptoms; and the resem-

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[4] Correspondence.

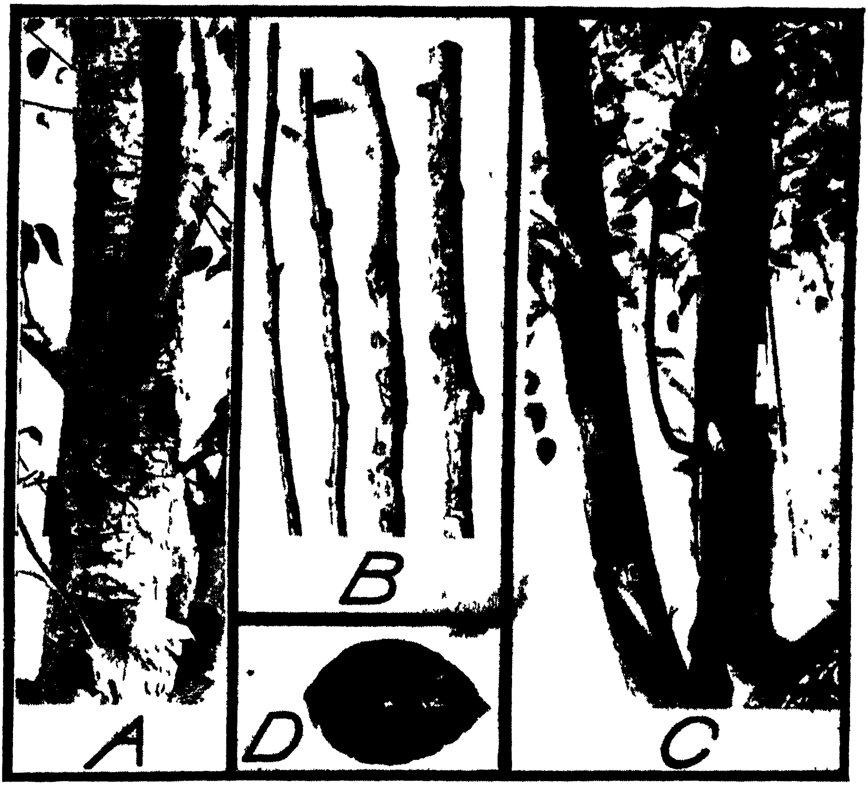

FIG. 2. Stony pit symptoms on pear trees. *A*. Natural bark cracking on healthy Bosc. *B*. Pimples appearing on young twigs (left), which crack to form target canker effects (right). *C*. Oak bark stage on Bosc scion above, with the Patten trunk below not yet showing bark symptoms. *D*. Veinlet chlorosis on Forelle leaf, a probable symptom of stony pit.

blance of the advanced phase to the bark of matured oak trees is very striking. Trees reaching this stage of the disease exhibit less foliage because of the failure of lateral buds to grow. Terminal shoots and fruit spurs bear most of the scant leaf surface.

In some cases the oak-bark condition may be found only on the scion wood of a topworked tree; in others, the rough bark may appear only on the trunk wood. It appears possible that the disease agent might be carried in either unit, in which case the bark symptoms may be transferred to the healthy bark only after several years' growth. This condition is illustrated in figure 2, *C*. Typical oak bark was produced on the diseased Bosc scion above, but the symptoms have not yet appeared in the Patten trunk stock below.

On the Leaves of Bosc: Two leaf symptoms have been observed on stony-pit trees. One consists of narrow, chlorotic areas along the veinlets of the leaves and the other of a faint mottling, especially on newly formed shoots. The definite association of leaf symptoms with the stony-pit disease has been complicated by the appearance of similar conditions on apparently healthy trees, and by the erratic presence of leaf symptoms on diseased trees. The veinlets chlorosis, however, appears to be the true leaf symptom of stony pit; but conditions that mask or favor its appearance are not yet understood. Leaves of the Forelle variety show a marked veinlet chlorosis or mottling (Fig. 2, *D*) which appears to be associated with a deformed and stony fruit. Proof that the disease of the Forelle and Bosc pear is the same, however, is lacking at present.

On Other Varieties: Stony pit is definitely known to occur in the Anjou pear. A roughened or partially deformed fruit results from the few to many shallow or deep pits occurring (Fig. 1, *B*). The pits are much shallower than in the Bosc on trees known to be naturally infected. Brown, necrotic spots or streaks generally appear within, or extend inward, from the pitted areas. The stony tissue is present below the pits in most cases. In contrast to the Bosc fruit, symptoms become noticeable on Anjou pears only a month or two before harvest. The resemblance to a condition in this variety, commonly attributed to a water deficiency, called drought spot, suggests the troubles may be the same.

Winter Nelis fruits often show symptoms indistinguishable from those on Bosc. Seckel, Easter, and Forelle pear fruits sometimes become rather deformed with various types of pitting accompanied by the internal stoniness. Patten and Old Home pears, produced on trees partially grafted to Bosc, occasionally form a few very shallow pits under which stone cells are present. That the symptoms in these varieties are those of stony pit has not yet been proved.

EXPERIMENTAL WORK

Soil-deficiency Studies: The symptoms of stony pit, as expressed in both the fruit and bark tissues of diseased Bosc pears, show considerable similarity to those in other plants known to be caused by the lack of certain soil elements. The similarity, particularly to drought spot and corky core in apple fruits,[5] and to a type of measles on apple bark,[6] suggested that a lack of boron might be responsible for the condition. Accordingly, boric acid, zinc sulphate, copper phosphate, and managanese borate, were injected into 20 diseased trees by the method employed by McLarty.[5] Additional diseased trees received soil applications or sprays of borax or boric acid. A decrease in the fruit pitting resulting from chemical treatments was not established over a period of 3 years. Boron, however, appeared to slightly improve the foliage size and color on several of the trees.

Relation to Insects: It was already mentioned that individual fruit pits sometimes resemble an injury caused by the tarnished plant bug. The insect injury, however, has never been seen in a severe form comparable to stony pit, and varieties unaffected by stony pit are susceptible to the insect injury.

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[5] McLarty, H. R. Tree injections with boron and other materials as a control for drought spot and corky core of apple. Scient. Agr. 16: 625–633. 1936.

[6] Young, H. C. and H. F. Winter. The effect of boron, manganese, and zinc on the control of apple measles. Ohio Agr. Expt. Sta. Bimonthly Bull. 22: 147–152. 1937.

Other insects in sufficient numbers to cause a fruit deformity as severe as stony pit, have not been observed on the pear trees.

Attempts to Culture Possible Causal Organisms: Several hundred isolations from diseased bark and fruit tissue have been made. The usual saprophytic forms of fungi and bacteria often were isolated from broken bark tissue, but these were to be expected. Cultures from diseased fruit tissue and from the unbroken bark pimples on diseased twigs have consistently remained sterile.

Experiments to Test Possible Virus Origin: On April 10, 1936, 2 healthy trees each of the Bartlett, Bosc, and Anjou varieties were budded with typically diseased Bosc material. In 3 additional trees of each variety, diseased buds were inserted in August of the same year. Five or more diseased buds were usually placed on the terminal shoots of an individual limb. In a few cases single buds were inserted in selected laterals or in different branches throughout the tree. Records of the fruit condition on the budded trees in 1937 and 1938 are shown in table 1.

The data presented in table 1 clearly show that the disease was transmitted from diseased buds to healthy Bosc and Anjou trees, but that the symptoms in most cases were delayed until the second season. A single stony pit fruit was observed near the diseased buds on one Bosc and Anjou limb each in 1937. Progress of the transmissible agent was thought to be observed from 4 to 12 inches below diseased buds during the first season, in the form of slight leaf mottling. The symptoms were very indefinite, however, and not present in all cases. Pitted fruit appeared at a distance of 2 to 20 feet from diseased buds in 1938. The most heavily pitted fruit appeared near the diseased buds and became less severe at a distance from them. Fruit pitting was especially noticeable on smaller limbs on which several diseased buds had been inserted at the terminal ends. The disease was typical in all respects to that occurring naturally on Bosc fruit. Transmission in the Anjou variety was very striking and the fruit pitting occurred in a more aggravated form than in the one orchard known to be naturally infected. From the severely pitted fruits (Fig. 1, *B*) near the diseased buds, the fruits became less pitted at a distance from them. In two cases, however, the virus had moved from the diseased buds through the budded limbs and into the main leader branches, on which pitted fruit appeared 20 feet from the diseased buds. Clean fruit remained at the tops of these branches at the harvest period.

Several young Bosc orchards have been observed in which a large percentage of the trees were diseased. In each case the original scion wood was obtained from infected orchards. Field observations tend to indicate that a slow spread of the virus occurs from tree to tree by means other than budding. Results of studies with this disease could be obtained much more rapidly if a variety showing definite and consistent leaf symptoms could be discovered.

TABLE 1.—*Virus transmission from diseased Bosc to healthy pear trees*[a]

Healthy variety budded	Row number	Tree number	Buds growing	Fruits—1937		Fruits—1938	
				With stony pit	Pit free[c]	With stony pit	Pit free
			No.	*No.*	*No.*	*No.*	*No.*
Bosc	9	1	10	0	390	17	578
	9	4	10	0	1170	97	1341
	9	6	10	1[b]	1495	66	1689
	I	6	10	0	260	48	26
	D	22	8	0	358	35	290
Anjou	B	22	10	0	60	60	16
	P	1	9	0	180	2	57
	17	4	10	1[b]	900	319	124
	17	5	10	0	1440	182	290
	0	4	10	0	180	8	41
Bartlett[c]	A	22	9	0	840	0	384
	8	22	9	0	420	0	340
	19	1	0	0	12	0	40
	6	6	10	0	750	0	440
	6	5	10	0	300	0	440

[a] Trees budded in 1936.

[b] Fruit pit near diseased bud.

[c] Number of fruits estimated from box yield.

CONTROL

Little can be offered at this time on control measures to employ against stony pit. Bosc trees having the oak-bark stage of the disease might best be eliminated from the orchard, since they are no longer profitable. Whether less severely diseased trees might profitably be topworked to Bartlett or other varieties, which appear to be either tolerant to or immune from the virus, remains questionable. Bartletts topworked on diseased Bosc trees have produced healthy fruit for 5 consecutive years, but their future performance needs watching. Even if the Bartlett fruits continue to be healthy, the possibility of the tree being a symptomless carrier needs consideration. Nurserymen should be very careful in selecting Bosc scion wood, since the disease is easily spread by this means. Knowledge of the varietal reactions of pears toward stony pit, and a test plant for identification purposes are urgently needed.

SUMMARY

Stony pit is described as a virosis of pear trees. The symptoms consist of a fruit pitting, a so-called oak-bark effect, and probably of a veinlet chlorosis of certain leaves. The trouble is known to be present on the Bosc variety and occasionally on the Anjou in California, Oregon, and Washington.

The disease was readily transmitted to healthy Bosc and Anjou trees from diseased Bosc buds; in most cases the symptoms appeared during the second season. The Bartlett variety was found to be either tolerant or immune by the same method. Control measures have not been studied.

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STUDIES ON A CULTURAL VARIANT OF RHIZOCTONIA SOLANI[1]

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(Accepted for publication November 2, 1938)

That cultural variation in *Rhizoctonia solani* Kühn is not common is evidenced by the fact that it has been reported only recently but not studied by Ullstrup.[2] The phenomenon has been observed only a few times by the writer, in a period of about 5 years.

The present investigation was undertaken to study the morphology, physiology, and pathogenicity of a sector formed in a culture growing on potato-dextrose agar and that was sharply delimited from the parent culture (Fig. 1).

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[1] The data presented in this paper were obtained in cooperative investigations by the Division of Sugar Plants, Bureau of Plant Industry, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station. Paper No. 1649 of the Journal Series of the Minnesota Experiment Station.

[2] Ullstrup, Arnold J. Leaf blight of China aster caused by *Rhizoctonia solani*. Phytopath. 26: 981–990. 1936.

It is recognized that some doubt may be cast upon the nature of this sector, since its parent colony was a tissue culture. That the sector is a true variant and not a result of a mixed culture is supported by the fact that the parent culture has been subcultured between 25 and 30 times and grown on various media and under a wide range of environmental conditions during a period of 5 years without the occurrence of either patch- or wedge-shape sectors.

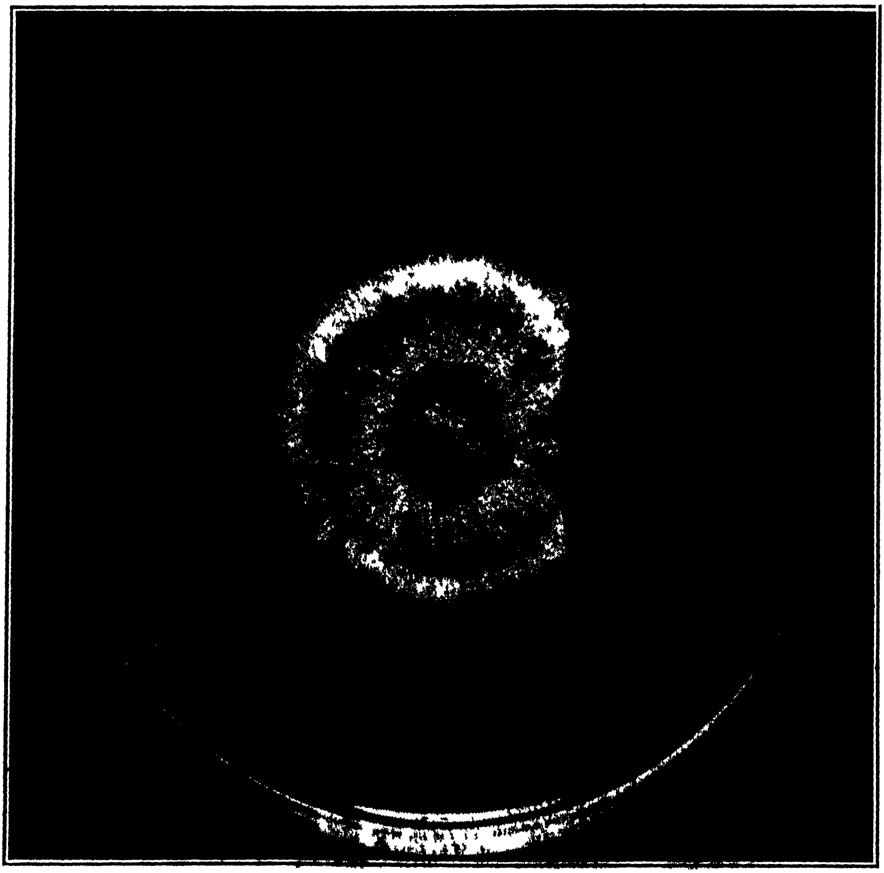

FIG. 1. Colony of sugar-beet isolate of *Rhizoctonia solani* showing large variant used in present studies.

CULTURAL CHARACTERS

Differences in cultural characters were studied on potato-dextrose agar and on a medium with a low carbon-nitrogen ratio. Cultural appearances were noted 18 days after inoculation of Petri dishes.

From the evidence presented in table 1, it appears that the variant is characterized by scant growth of mycelium on potato-dextrose agar as compared with dense and uniform growth of the parent culture. On the low carbon-nitrogen agar, a slight difference in color is the only character that distinguishes the two cultures.

TABLE 1.—*Cultural characteristics of the variant and parent cultures of Rhizoctonia solani on two different artificial media 18 days after inoculation*

Culture	Medium	
	Potato dextrose	Low carbon-nitrogen ratio
SB–28 (Parent)	Colony Prant's Brown,[a] margin regular; growth uniform and dense; concentric rings present. No sclerotia produced	Colony Light Buff; margin slightly irregular; growth very scant, fine. No sclerotia produced
SB–28–1 (Variant)	Colony Sayal Brown; margin irregular; growth scant, fine; no concentric rings. No sclerotia produced	Colony Ivory Yellow; margin irregular; growth scant, fine. No sclerotia produced

[a] Colors are according to Ridgway's color standards.

MORPHOLOGY

To ascertain whether the variant was different in size of mycelium from the parent, a study was made of the hyphal diameters of each. The cultures were grown on potato-dextrose agar in Petri dishes for 5 days at about 25° C. The hyphae were then mounted in a 10 per cent aqueous solution of glycerine on glass slides. By means of a projection apparatus the images were thrown on a white background and measured. Measurements were made to the nearest tenth of a millimeter with a vernier-caliper of 200 hyphae of each culture.

It was found that the mean diameter of the hyphae of the parent culture was 7.2 μ, whereas, of the variant, it was 6.9 μ. This difference in size is not significant since the value of "F" in the analysis of variance did not reach the 5 per cent point.

PHYSIOLOGY

Effect of Medium on Rate of Growth

Comparative tests of the 2 cultures were made to determine radial growth, as measured by size of colony, on six different media. Two experiments were made in 1935 and one in 1936. In each experiment the cultures were grown on 5 Petri dishes of each medium. The measurements were made 91 hours after inoculation.

From the data given in table 2 it appears that marked differences in radial growth occurred between the parent and variant cultures on the same and different media. The variant grew more rapidly on the high-, medium-, and low-nitrogen media, whereas the parent culture grew more rapidly on methylene blue, potato-dextrose, and dextrose media. The radial growth of these cultures on 4 different media is illustrated in figure 2.

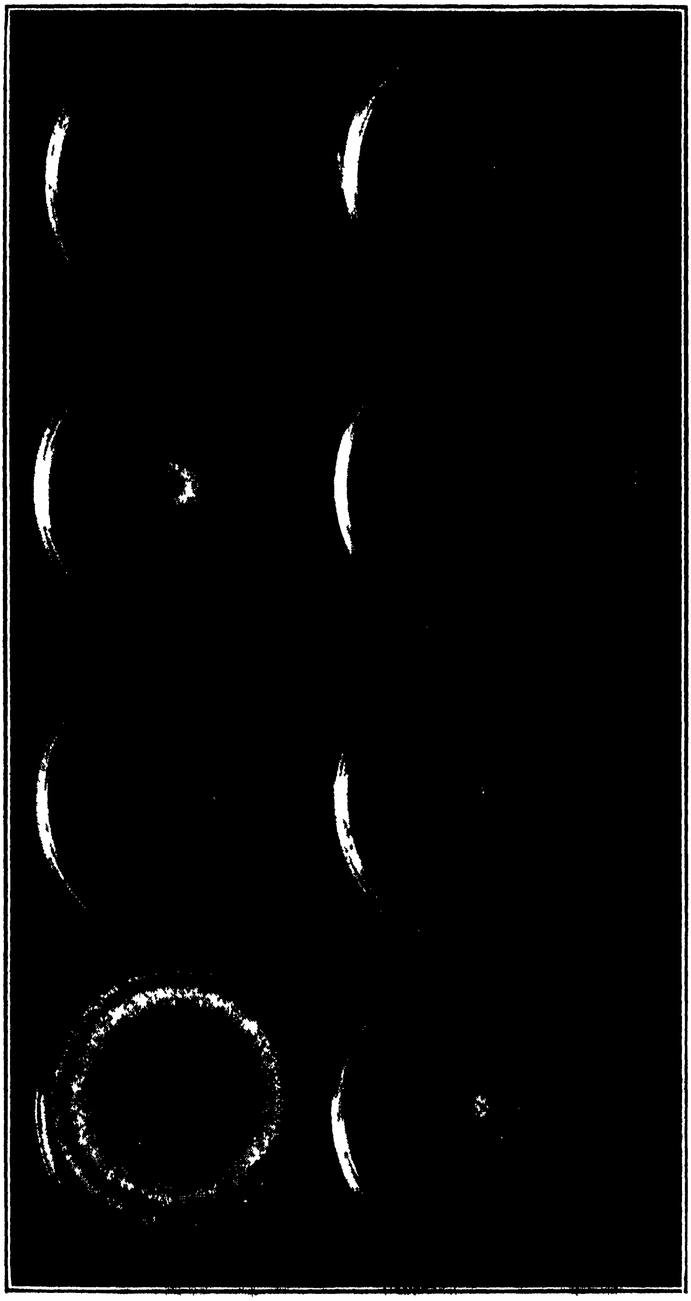

FIG. 2. Radial growth of variant and parent culture of *Rhizoctonia solani* on 4 different artificial media. Vertically, 4 dishes on left are inoculated with the parent culture and the 4 on the right with the variant. Horizontally, beginning at the top, the first 2 dishes contain a high-nitrogen agar; second 2 dishes contain a medium-nitrogen agar; third 2 dishes contain a low-nitrogen agar; the fourth 2 dishes contain potato-dextrose agar.

TABLE 2.—*Radial growth of variant and parent culture of Rhizoctonia solani on six different artificial media after 91 hours at room temperature in 1935 and 1936*

Culture	1935		1936	Average	1935		1936	Average
	Test No. 1	Test No. 2			Test No. 1	Test No. 2		
	High nitrogen				Medium nitrogen			
Parent	3.0	0.0	tr[a]	1.0	15.3	5.3	tr	6.9
Variant	18.3	4.2	5.8	9.4	37.7	18.8	14.0	23.5
	Low nitrogen				Methylene blue			
Parent	20.7	11.8	2.2	11.6	52.7	38.4	35.6	42.2
Variant	40.7	23.8	19.6	28.0	24.3	21.4	7.4	17.7
	Potato-dextrose				Dextrose			
Parent	85.3	82.2	74.2	80.6	82.7	51.8	63.4	66.0
Variant	40.7	35.0	17.6	31.1	37.3	18.2	24.6	26.7

[a] Tr = trace, considered 0.1 mm. in determination of means.

Effect of Various Amounts of Sucrose on Growth

An experiment was made to determine if there was any difference in radial growth of the variant and parent culture on artificial media containing different amounts of sucrose. Potato agar without sucrose and with 1, 5, 10, and 20 per cent sucrose was used. The Petri dishes, inoculated in triplicate, were maintained for 73 hours at room temperature before making measurements.

It is evident, from the data in table 3, that the parent culture grew faster than the variant at 0, 1, and 5 per cent sucrose, but, at 10 and 20 per cent sucrose, the variant grew faster.

TABLE 3.—*Effect of different amounts of sucrose on the radial growth of variant and parent cultures of Rhizoctonia solani*

Culture	Percentage of sucrose and average radial growth in millimeters				
	0	1	5	10	20
Parent	65.7	59.7	33.0	17.0	7.3
Variant	8.0	17.6	26.0	30.0	19.0

Temperature Relations

Two experiments were made each consisting of 5 replications, to determine the radial growth of the variant and the parent culture on potato-dextrose agar, after 60 hours, at 15°, 23–25°, and 29–30° C.

TABLE 4.—*Effect of temperature on radial growth on potato-dextrose agar of variant and parent cultures of Rhizoctonia solani*

Culture	Temperature and radial growth in millimeters								
	15° C.			23–25° C.			29–30° C.		
	Test 1	Test 2	Ave.	Test 1	Test 2	Ave.	Test 1	Test 2	Ave.
Parent	14.2	19.6	16.9	42.8	35.6	39.2	45.2	52.4	48.8
Variant	7.6	2.6	5.1	24.2	11.8	18.0	28.6	26.0	27.3

The results (Table 4) indicate that the radial growth of the parent is much more rapid than that of the variant at each of the three temperatures. There is no differential response of temperature and radial growth for the 2 cultures. It is of interest to note that the ratio of growth of the parent to that of the variant decreases with the increase in temperature. The ratio is 3.3 at 15°, 2.2 at 23–25°, and 1.8 at 29–30° C.

Pathogenicity

That sudden changes occur in some isolates of *Rhizoctonia solani* is significant, and may be indicative of what actually occurs in nature. The most important question, however, is: Is this change in appearance and physiology accompanied by a corresponding change in pathogenicity?

Inoculation of Sugar-beet Roots

Inoculations of large sugar-beet roots were made with the variant and parent cultures of *Rhizoctonia solani* in both 1935 and 1936. The method of inoculation was the same as that previously used.[3]

From the results of these inoculations presented in table 6, it is apparent that in both years the variant was less aggressive than the parent. A comparison of the decay produced by each culture is shown in figure 3.

TABLE 5.—*Influence of soil temperature on the percentage of damping off of sugar beets as caused by variant and parent cultures of Rhizoctonia solani*

Culture	Soil temperature and percentage of damping off	
	15° C.[a]	25° C.[b]
Parent	73.4	69.4
Variant	100.0	100.0
Control	0.0	0.0

[a] Stand counts made 18 days after planting.
[b] Stand counts made 6 days after planting.

It is apparent from the data in table 6 that the relation of parasitic behavior of the variant to that of the parent culture had not changed appreciably during the period of a year. Likewise, the relationship, as regards radial growth on different media, between variant and parent had not changed after the lapse of a year (Table 2).

TABLE 6.—*Comparison of amount of decay of sugar-beet roots caused by variant and parent cultures of Rhizoctonia solani in 1935 and 1936*

Culture	Number of roots inoculated			Percentage of decay		
	1935	1936	Total	1935	1936	Average
Parent	8	17	25	38.7	27.6	33.2
Variant	8	17	25	1.6	15.4	8.5
Control	8	17	25	0.0	0.0	0.0

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[3] LeClerg, E. L. Parasitism of *Rhizoctonia solani* on sugar beet. Jour. Agr. Res. [U. S.] 49: 407–431. 1934.

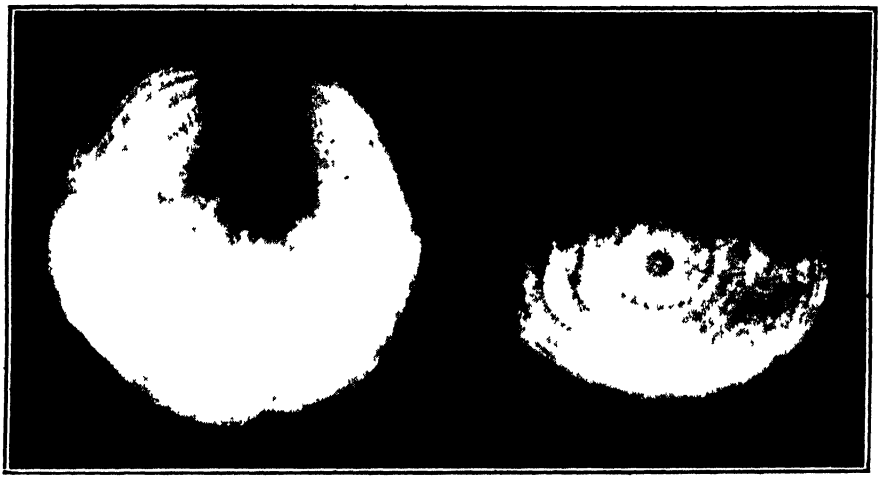

Fig. 3.—Comparison of decay caused by variant and parent culture of *Rhizoctonia solani* in roots of sugar beets in 1935. Decay produced in the root on the left is the maximum amount caused by the variant, whereas that produced in the root on the right is about the average amount caused by the parent culture.

Effect of Soil Temperature on Damping-off

The effect of soil temperature on damping off of sugar beets caused by the variant and parent cultures of *Rhizoctonia solani* was studied. Inoculum consisting of the fungus growing on sterilized oats and wheat grains was mixed with steamed soil and placed in 4-inch pots; the control consisted of steamed soil with only sterilized grains. The experiment consisted of 4 replications and each pot was planted with 35 seed-balls of sugar beets, which were subsequently maintained in incubators at 15° and 25° C., respectively.

It is of interest to note that the variant caused more damping off than did the parent (Table 5). The seedlings in the pots containing the parent culture at 15° C. were only about 25 per cent as high as those in the controls.

SUMMARY

A sector variant, which occurred in a sugar-beet isolate of *Rhizoctonia solani,* is compared with the parent culture in physiology, morphology, and pathogenicity.

The diameter of hyphae of the parent culture was not significantly different from that of the variant.

The parent culture grew faster than the variant on potato-dextrose, dextrose, and methylene-blue media, but much slower on media of high-, medium-, and low-nitrogen composition.

On media containing different amounts of sucrose, the parent grew faster at 0, 1, and 5 per cent, whereas at 10 and 20 per cent sucrose the rate of growth of the variant was more rapid.

The radial growth of the parent is greater than that of the variant at 15°, 23–25°, and 29–30° C.

The parent culture was more aggressive than the variant in causing root rot of large sugar-beet roots.

The variant caused more damping off of sugar beets at 15° and 25° C. than did the parent culture.

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A STUDY OF SUBERIN AND SUBERIZED DEPOSITS OF DISEASED POTATO TUBERS[1]

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(Accepted for publication December 2, 1938)

INTRODUCTION

Orton and Hill (11) in 1937 described a new disease of potatoes of unknown cause, which was prevalent and destructive in West Virginia. The name blue stem was applied to this disease because its most characteristic symptom is the production of an excessive amount of blue or purplish pigment in the stems of the Rural variety. Another typical symptom is the presence of a discontinuous dendritic necrosis of the tissues of the stem end of the tubers. The microchemical characters of these lesions have been described by Hill and Orton (7). Since the lesions appear to differ in nature from similar ones caused by the more common potato diseases, it was thought desirable to make a more detailed comparison of the stem-end necrosis associated with blue stem with that caused by certain other diseases. This paper presents the results of a microchemical study of suberin and suberized deposits in the necrotic lesions of tubers affected with blue stem as compared with: (a) tubers from plants affected with *Fusarium avenaceum* from Wisconsin; (b) tubers from plants affected with "purple-top wilt," a disease of unknown cause from Minnesota; tubers from Maine affected with (c) net necrosis and (d) "stem-end browning" as described by Folsom, Libby, Simpson, and Wyman (3); and tubers from West Virginia affected with hollow heart. Diseased tubers were obtained through the courtesy of John G. McLean of the Wisconsin Agricultural Experiment Station, J. G. Leach of the Minnesota Agricultural Experiment Station, and A. E. Rich of the Maine Agricultural Experiment Station.

A well-defined wound periderm was observed surrounding the necrotic lesions in some of the material. A study of the microscopical and optical properties of wound cork in various stages of its development has been included here although no significant difference was observed between the wound periderm of the different kinds of material.

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[1] Published with the approval of the Director, West Virginia Agricultural Experiment Station, as Scientific Paper No. 208.

METHOD AND MATERIALS

A summary of the microchemical test for suberin is given by Molisch (10), Tunmann (16), and Frey-Wysseling (4). Suberin and suberized deposits are insoluble in 75 per cent sulphuric acid, 50 per cent chromic acid, and copper-oxide-ammonia; they are soluble in potassium hydroxide when boiled for a short time, and in Schultze's reagent. The color reaction is a sulphur yellow with dilute potassium hydroxide and red with Sudan III. Suberin is anisotropic, and suberized deposit is isotropic. Suberized deposits give a cherry-red color with Millon's reagent and a reddish-brown with Lieberman's reagent; this is a positive test for phenol. Walls containing suberin give a negative test for phenol. Gyaiaconic acid gives a blue color; after 15 minutes in 1 per cent solution of benzidine in alcohol, the cells containing oxidase become blue. These more common microchemical tests were supplemented by a study of the optical properties of the tissues as revealed by a petrographical microscope. Under the petrographic microscope suberin and cutin are length-fast, while cellulose is length-slow. With the gypsum plate the blue of the cellulose wall is parallel to the slow ray; this indicates length-slow. The blue in the suberin and cutin is transversed with the slow ray of the gypsum plate, and is said to be length-fast.

Tubers used for a study of wound cork formation were cut and placed in moist chambers at various temperatures and examined at regular intervals to note the formation of cork cambium. Materials were fixed in formol-acetic alcohol, dehydrated and embedded in paraffin, then cut 10 μ in thickness. The paraffin was removed with xylol and mounted in balsam, while other slides were stained with Flemming's modified triple stain. Free-hand sections also were made and studied extensively.

MICROCHEMISTRY OF WOUND CORK FORMATION

Rhodes (14) discussed in detail the anatomy and the chemical nature of potato cork; it has been described by a number of other workers as a protective layer against water loss, invading fungi, and bacteria. The chemical changes associated with cork formation involve breaking-down and building-up processes. Haberlandt (6) concluded that cell division occurred only in the presence of a fragment of a vein containing phloem tissue; later, however, he showed that cell division was promoted by the presence of crushed cells. According to the observations of the writer, cell division may occur in the absence of phloem. In blue-stem necrosis, cell division failed to occur near the necrotic phloem, although normal cork was formed in the absence of phloem tissue. The first observable chemical change was the breaking down of the starch into glucose (Fig. 1, C), with a marked increase in the amount of protoplasm. Starch grains near the region of the cut surface were covered with a suberized deposit. This deposit was observed on the cellulose walls after 1 or 2 days; Rhodes calls this material suberin, but in the writer's observation it did not react positively to suberin tests. Glucose, fat, proteins, tyrosine, and nitrates occurred in higher concentrations in the region of cell division. When sections were treated with sulphuric acid the cellulose was hydrolyzed after 3 hours, leaving the isotropic suberized deposit and anisotropic suberin (Fig. 2, A, B, and C). Transverse sections

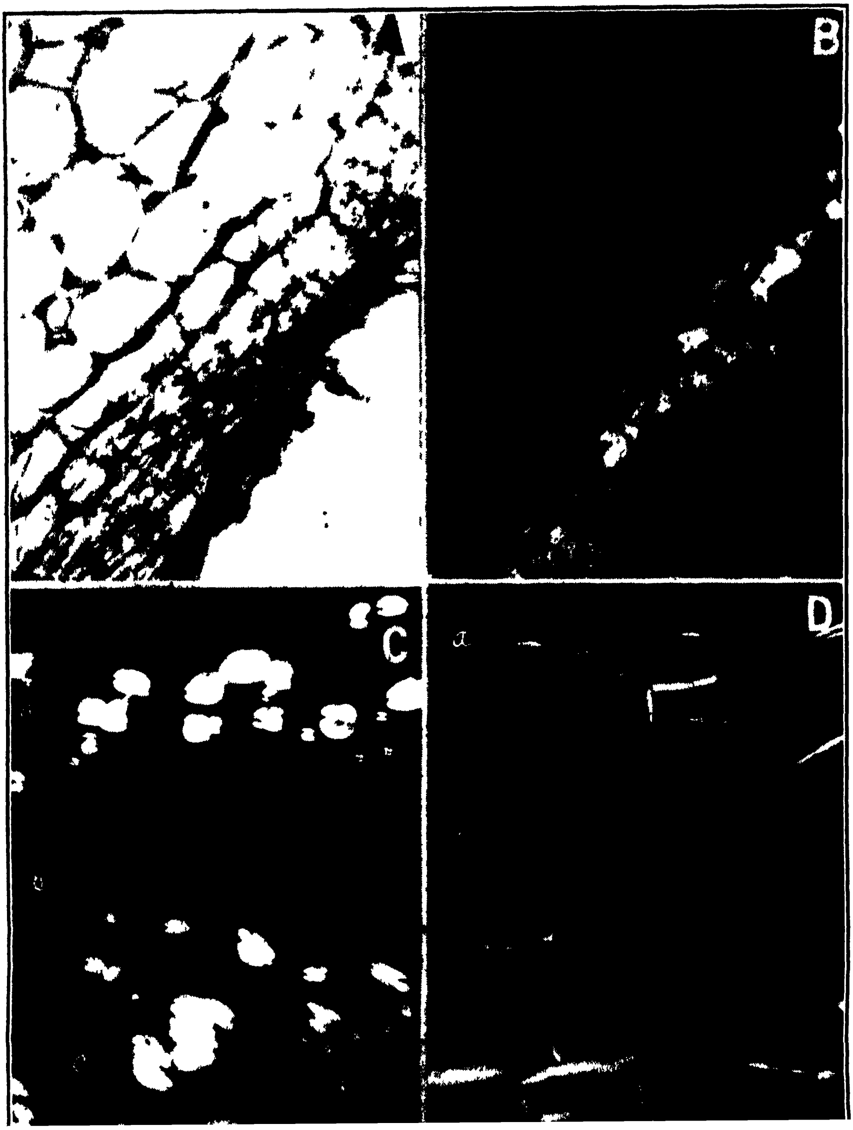

FIG. 1. A. Transverse section of wound periderm of potato tubers after treating with sulphuric acid for 5 hours; cellulose hydrolyzed. ×160. B. Same with polarized light, showing anisotropic periderm (lower right) and isotropic suberized deposit on the walls of the larger cells (upper left). ×140. C. Starch hydrolysis associated with wound periderm; *a*, starch grains adjoining the cut surface; *b*, region of starch hydrolysis and cambium formation; *c*, starch in normal parenchyma cells. ×115. D. Wound periderm treated with Schultze's reagent for 4 hours; *a*, cellulose walls adjoining the cut surface; *b*, region of cell division. ×160.

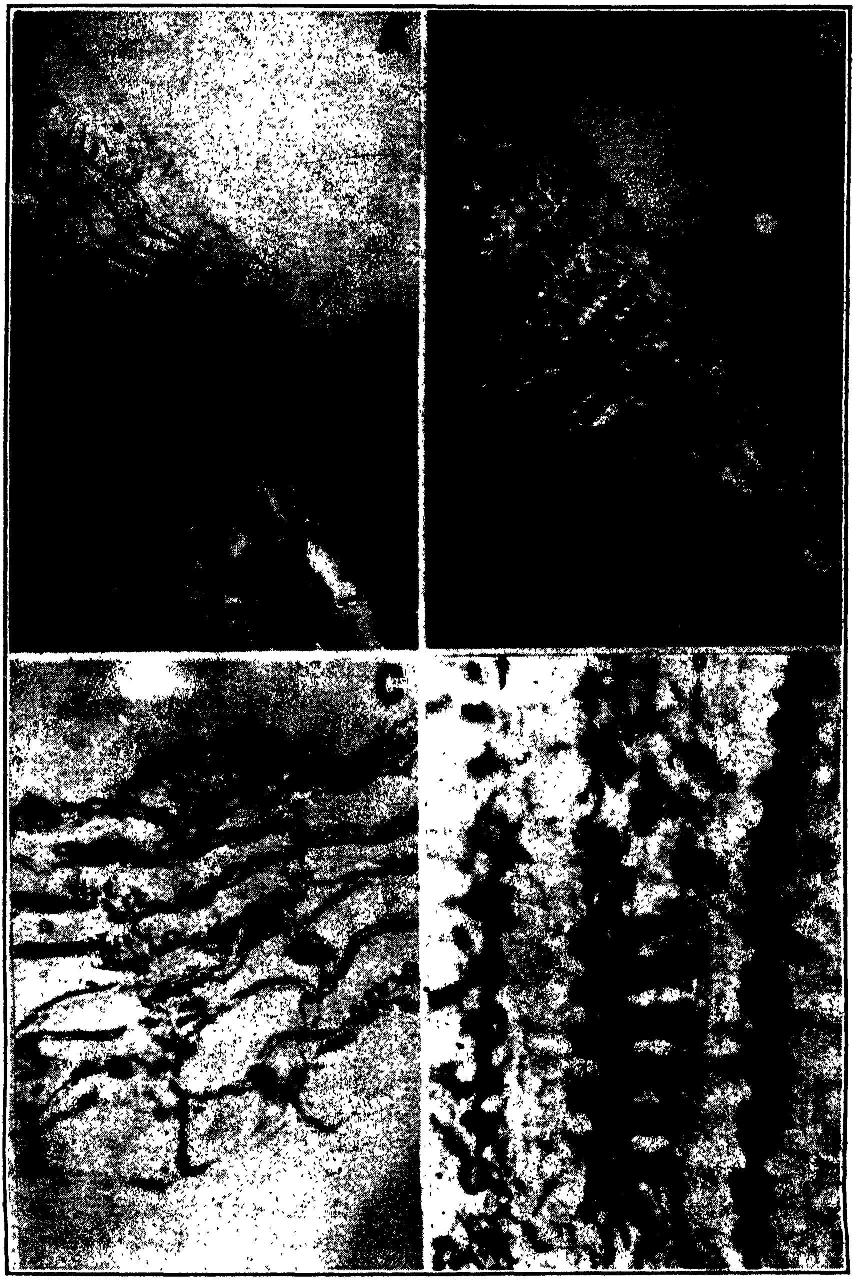

FIG. 2. A. Wound periderm treated with sulphuric acid for 3 hours to dissolve the cellulose. × 130. B. Same with polarized light showing the anisotropic suberin walls of the wound-cork cells; *a*, isotropic suberized deposit on the original cell walls. × 120. C. Normal periderm treated with 75 per cent sulphuric acid for 24 hours showing the suberin walls in the periderm and the absence of suberized deposits. × 560. D. Longitudinal sections of lesions in tubers affected with purple-top wilt after treating with 75 per cent sulphuric acid for 4 hours showing the suberized deposit on the vessel walls and spirals. × 825.

of wound cork, after treatment for 5 hours with sulphuric acid, showed isotropic suberized deposits in the cells adjacent to the wounded surface and anisotropic suberin in the cell walls of the wound periderm (Fig. 1, A and B). Phenol was detected in the suberized deposit. A large amount of oxidase was found associated with the wound periderm.

When the cork cells begin to form from the meristem they give a positive test for cellulose (Fig. 1, D), and when the periderm has completely formed they give a positive test for cellulose and suberin (Fig. 2, A and B). Treatment of wound periderm for 4 hours in Schultze's reagent removed the suberin and suberized deposit, leaving the cellulose (Fig. 1, D). Treated with 75 per cent sulphuric acid, the cellulose is hydrolyzed. Suberized deposits and walls containing suberin give a positive test for cerin.

SUBERIN IN NECROTIC TISSUES

Hill and Orton (7) have reported the microchemical properties of the abnormal tissues, and the suberized deposit associated with the various types of necrosis in blue stem. It was shown that cellulose and pectic substances were partially masked by a deposit of suberin in the necrotic regions of the phloem and parenchyma, and that cellulose and lignin were present in the walls of necrotic xylem. A suberin-like substance, soluble in Schultze's reagent and giving a positive test for cerin, was found in necrotic phloem, xylem, and parenchyma. Phenol also was detected in the walls of the necrotic areas.

Tubers from Wisconsin affected with *Fusarium avenaceum,* a vascular parasite of potato, showed a precipitate of suberized deposit in the phloem and xylem (Fig. 3, C and D). Necrotic phloem groups were surrounded by a well-defined layer of wound cork. Sections treated in sulphuric acid for 4 hours showed hydrolysis of the cellulose, leaving the suberin and a suberized deposit (Fig. 3, A and B). The suberin cell walls were birefringent.

Tubers from Minnesota affected with "purple-top wilt" showed a vessel necrosis with a suberized deposit upon the vessel walls. Longitudinal sections through vessels treated with 75 per cent sulphuric acid for 4 hours showed the presence of a suberized deposit in the spirals and other vessel walls (Fig. 2, D). No definite wound cork was observed around the necrotic region. A material similar to the suberized deposit was precipitated in cells adjoining the necrotic vessels.

Tubers from Maine affected with stem-end browning showed a phloem and xylem necrosis with a suberized deposit on and within a group of necrotic cells. This necrotic region was surrounded by a wound cork. The lesions resembled in some respects those associated with leaf roll but differed in certain important respects. Artschwager (2) described the necrotic region of the stem in leaf roll as a deposit of globular masses, yellow in unstained sections, insoluble in mineral acids, and increasing in color with alkalies. With phloroglucinol and hydrochloric acid, this deposit gave a negative test for lignin. A reddish color was obtained with alcarin; this suggested the presence of cutin or cutin-like substances. The birefringence of the walls in the

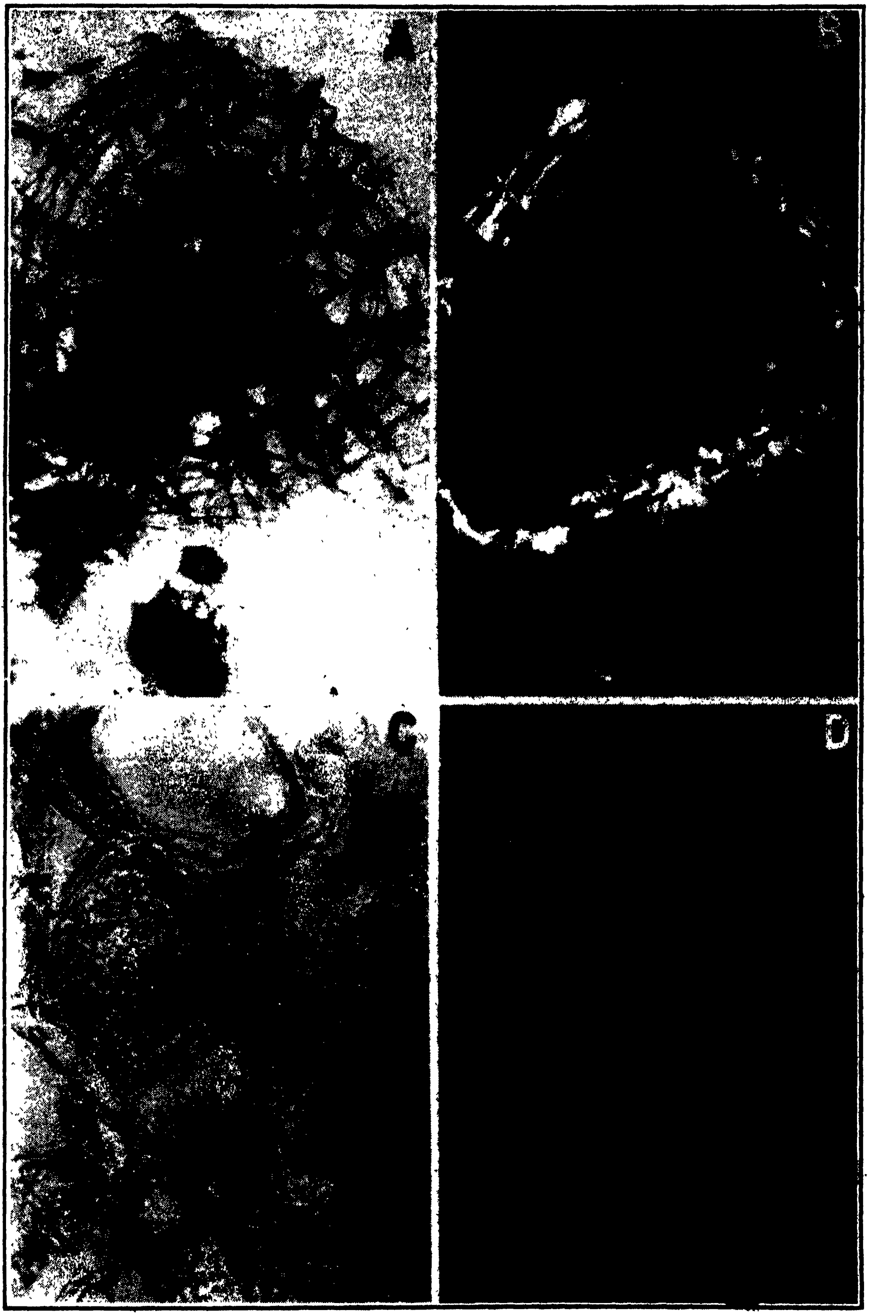

FIG. 3. A. Sections of potato tubers affected with *Fusarium avenaceum* after treating with sulphuric acid for 4 hours to show the suberized deposit and the suberin of the surrounding cork. ×130. B. The same with polarized light showing the anisotropic suberin and isotropic suberized deposit. ×120. C. Suberized deposit in xylem affected with *F. avenaceum*. ×860. D. Same with polarized light showing the anisotropic cellulose in the xylem walls and the isotropic deposit within the vessels. ×600.

necrotic lesions of stem-end browning indicated the presence of suberin rather than cutin. The cutin of a number of different plants examined by the writer showed a birefringence different from that shown by the walls in the necrotic lesions. The optical properties of cutin observed by the writer agree with those reported by Ambronn (1).

Tubers from Maine affected with net necrosis showed a phloem necrosis with a suberized depósit that masked the phloem cells. Gilbert (5) indicated that cutin and suberin were present in wound periderm and that lignin was present in the necrotic areas. His observations concerning the reddish-violet color in the yellow necrotic center when treated with phloroglucinol and hydrochloric acid were confirmed. However, the lignin of the vessels does not take the same reddish violet but a brick red tinge, easily distinguished from the color in the necrotic areas. The reddish violet obtained by Gilbert in the necrotic areas may be caused by phenol. The necrotic areas gave a positive test for phenol, whereas the lignified walls of the vessels gave a negative test.

A cork layer occurs around the cavity in hollow heart of potato; it is similar to that of wound periderm, but is much more spongy and has a depth of 2 to 3 times that of wound periderm (Fig. 4, D). There was only a small amount of suberized deposit associated with this type of injury as compared with the amount of the suberized deposit in wound periderm. Starch grains were absent in the region of the cork, but occurred normally in the adjoining tissues. The cell walls of the cork layer are composed of cellulose and suberin, with the cellulose masking the suberin, as revealed by the polarizing microscope.

SUMMARY

Methods are presented for determining the difference between suberin and suberized deposit found in lesions in potato tubers affected with several different diseases. The petrographical microscope has been used along with various microchemical tests to determine the presence of suberin and cellulose in the cell walls of potato tubers. The suberin is anisotropic in normal periderm, in wound periderm, and in corky cells surrounding the necrotic areas. The suberized deposit is isotropic and is laid down on, or may be infiltrated in, a cellulose matrix and is the result of oxidation and condensation of cell sap drying on cellulose walls of cells associated with wound periderm, and with necrosis in the potato tuber. The suberized deposit has been studied in blue stem, purple-top wilt, net necrosis, hollow heart, stem-end browning, and in potato tissue invaded by *Fusarium avenaceum*. In all of the foregoing cases this deposit has the same chemical and physical properties. Cutin is not associated with necrosis in the diseased potato tubers. There is only a small amount of suberized deposit associated with hollow heart, as compared with that associated with wound cork. The cork cambium formed in hollow heart is spongy, whereas that of normal periderm is not. The suberized deposit always masks the anisotropic properties of starch, cellulose, and suberin. Phenol is associated with the suberized deposit,

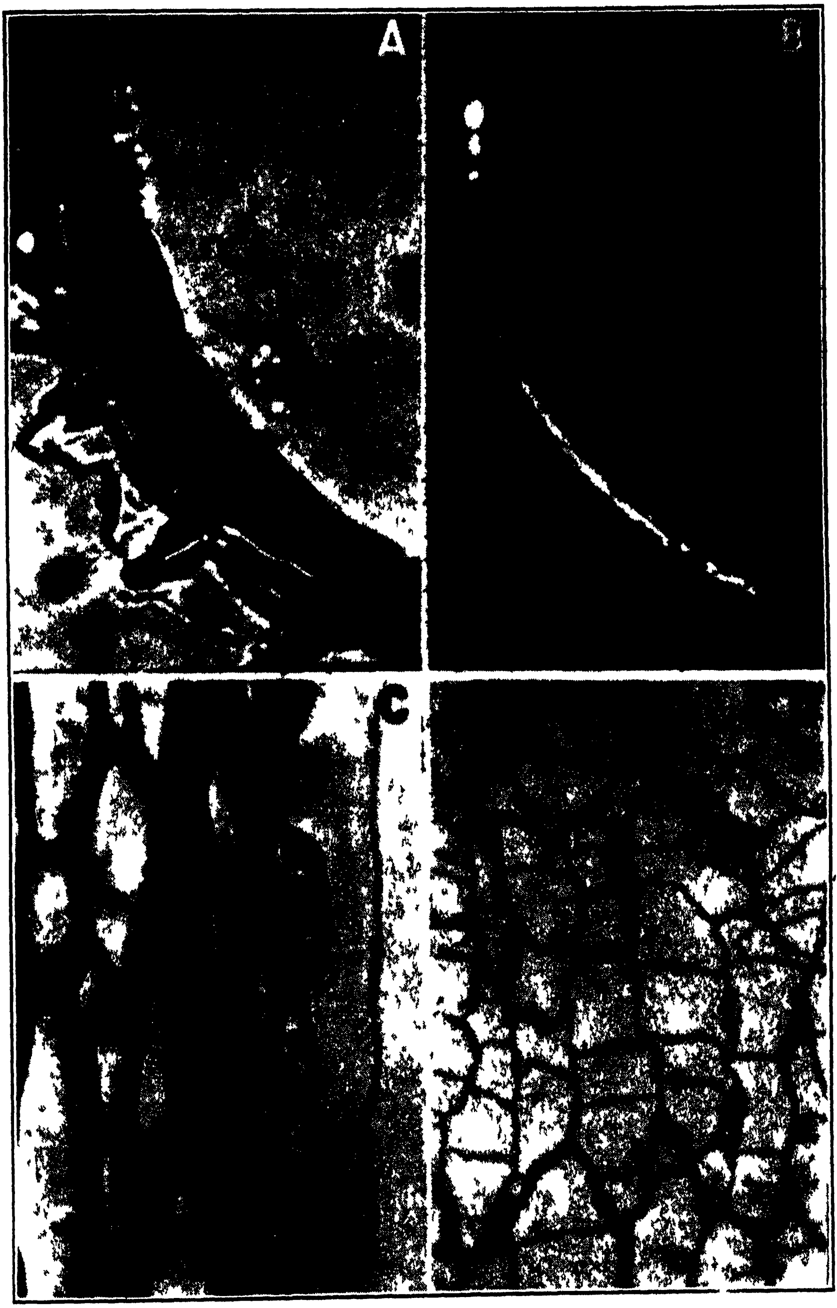

FIG 4 A Cuticle of Rome Beauty apple treated with sulphuric acid for 3 hours to remove cellulose and pectin; the cuticle curved backward after the treatment. ×660 B. Same with polarized light showing anisotropic cutin. ×608. C Sections of Rome Beauty apple stained with Flemming's modified triple stain, *a*, unstained cutin. ×550. D. Sections of periderm of hollow heart showing spongy cork cells and absence of starch; *a*, region of suberized deposit. ×550.

but is absent in suberin and in cellulose walls. Suberin and cutin are length-fast and cellulose is length-slow. Optical properties distinguishing cutin, suberin, suberized deposits, and cellulose have been found useful in the study of the necrotic lesions.

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POWDERY MILDEWS OF PEACH AND ROSE[1]

C. E. YARWOOD

(Accepted for publication October 11, 1938)

Powdery mildew of peach and that of rose, each caused in part, at least, by strains of *Sphaerotheca pannosa* (Wallr.) Lév., are common and do considerable damage in certain sections of California.

Peach mildew overwinters in part, at least, in infected peach buds, and such buds show systemic infection when they develop in the spring. This has been demonstrated only with heavily infected material in Berkeley. From 57 dormant twigs that were surface-sterilized and placed in plugged tubes on January 28, 1936, were formed 17 mildewed and 112 healthy leaf shoots by February 18. In commercial orchards such infected buds are very rare, but may be conspicuous on heavily infected seedling plants (Fig. 1, A and B). Peach mildew develops slowly on the foliage in the spring, but as high as 97 per cent fruit infection was observed when the fruits were about the size of walnuts on May 31, 1938. In late summer and fall the disease becomes conspicuous as a white powdery mass on the young foliage of non-treated orchards, usually in patches on the lower epidermis causing chlorotic spots and puckering of the leaves. It also occurs as a felty mass on young twigs, especially water sprouts. Heavily infected twigs may be killed during the winter.

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[1] The assistance of nontechnical employees of the Federal Works Progress Administration is acknowledged.

Powdery mildew of the rose may overwinter by at least 2 methods in addition to the probable function of perithecia. Active mildew may persist on the few leaves that live through the winter in mild seasons, as was observed in the winter season of 1934–35 in Berkeley, or it may overwinter in the buds as does peach mildew (Fig. 1, C). The evidence for the overwintering of rose mildew in the buds is circumstantial only, as no infected dormant buds have been forced under controlled conditions.

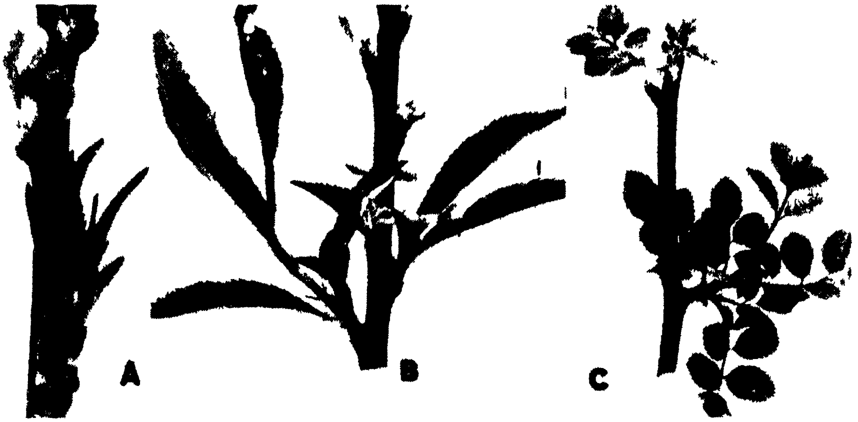

FIG. 1. Bud infection caused by peach and rose mildew. A. Twig of Palora peach from orchard at Gilroy, California, March 28, 1938; lower bud healthy, upper bud mildewed. B. Twig of seedling peach from Berkeley, April 4, 1938; two lower buds healthy, three upper buds mildewed. The systemic mildew infection has reduced leaf growth. C. Twig of *Rosa odorata* from Berkeley, March 15, 1938; lower bud healthy, upper bud mildewed.

That *Sphaerotheca pannosa* of peach and of rose are different is indicated by the failure of cross-inoculations, though peach mildew made considerable mycelial growth on the lower surfaces of detached leaflets of Briarcliff, Belle of Portugal, and Pernet roses on 10 per cent sucrose solution. Inoculations with rose-mildew conidia on peach showed no growth beyond germination and cell penetration. Conidiophores of the mildews on peach and rose were similar in gross morphology, but the conidia of rose mildew were slightly larger. Ninety conidia from peach leaves averaged 23.4 13.4 μ, and 90 conidia from rose leaves averaged 27.4 × 14.2 μ.

On ornamental peaches, powdery mildew is less common than on commercial fruiting peaches, but the luxuriant development of powdery mildew on leaves of ornamental peaches infected with leaf curl (*Taphrina deformans* (Fckl.) Tul.), has been very marked during the years 1935–38. Curl-infected leaves of commercial peaches usually die by midsummer, and powdery mildew has not been conspicuous on them, though present in small amounts. Curled leaves of ornamental peaches, on the other hand, may live in a vigorous state until October, as observed in 1937 and 1938, and such leaves may be heavily infected with mildew while noncurled leaves are free. On September 5, 1937, the mildew infection was determined on 1 random curled shoot and on 1 random noncurled shoot on each of 13 ornamental peach trees. Of 88 curled leaves, 84 were infected with mildew, while of 96 noncurled leaves, none was infected with mildew. In 1938 mildew occurred to a slight extent on the noncurled leaves of these same 13 trees, but was much more severe on the curled leaves. Microscopically, the mildew occurring on curled peach leaves is similar to that on noncurled leaves of commercial and ornamental peaches, and the haustoria are confined to the hypertrophied epidermal cells of the curled leaves. This greater development of peach mildew on curl-infected leaves than on healthy leaves is somewhat similar to the greater development of leaf rust on mildew-infected than on nonmildewed tissues as reported by Johnston.[2]

SUMMARY

Sphaerotheca pannosa on peach and *S. pannosa* on rose were found to be morphologically and pathologically distinct, and each was found to overwinter in infected buds.

Sphaerotheca pannosa on peach developed more luxuriantly on leaf-curl-infected leaves of ornamental peaches than on noncurled leaves.

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PHYTOPATHOLOGICAL NOTES

Tripsacum dactyloides, Another Native Host of Aplanobacter stewarti.—In September, 1938, bacterial wilt lesions resulting from *Aplanobacter stewarti* (E.F.S.) McCulloch, were found developing under natural conditions on *Tripsacum dactyloides* (L.) growing on the Potomac River bottom plots on the Arlington Experiment Farm, Arlington, Virginia. These plants were grown from seed obtained from the Soil Conservation Service as their No. T77, collected at Bellsville, Texas, in 1936. The seed was planted in early June, 1938, adjoining a plot of susceptible sweet corn infected with bacterial wilt. The plants came up promptly and, by September, had grown into leafy rosettes about 2 feet in diameter. No inoculations were made but flea beetles, *Chaetocnema pulicaria* Melsh., were observed feeding on the leaves, and feeding injuries of these beetles were common. On September 23 a few brown streaks, 1 to 3 mm. wide and 1 to 3 inches long, were found on the leaves, which, by transmitted light, had a somewhat water-soaked appearance. Bacteria streamed in abundance from sections of these lesions under the microscope. On plates poured from the lesions pure cultures of yellow colonies developed that produced typical bacterial-wilt symptoms on susceptible sweet corn (8482 × 14 from Glenn M. Smith).

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[2] Johnston, C. O. The effect of mildew infection on the response of wheat-leaf tissues normally resistant to leaf rust. Phytopath. 24: 1145–1146. 1934.

Tripsacum dactyloides (eastern gamagrass) occurs throughout the eastern United States from Massachusetts west to Iowa and Nebraska, south to Florida and Texas. It also occurs in the West Indies and from Mexico to Brazil.[1] There are, however, two forms of *Tripsacum dactyloides,* one known as the Texas Tripsacum with a haploid chromosome number of 18 and a Connecticut form with a haploid chromosome number of 36.[2] The plant on which infection occurred was examined by Virginia H. Rhoades, who found that the somatic cells contained the diploid number of 36 chromosomes characteristic of the Texas form of this species. The distribution of the two forms apparently is not known.

Repeated inoculations of young shoots and leaves of *Tripsacum dactyloides,* occurring naturally on the Arlington Farm and of 3 other species of *Tripsacum* grown in the field and in the greenhouse, have given only negative results and no natural infections have previously been observed in the field. These species of the genus *Tripsacum* have therefore been reported as immune from wilt.[3]

The wilt lesions found on *Tripsacum dactyloides* during the past season are small and inconspicuous; but, since they developed in the field from natural sources of infection, it is likely that they occur to some extent each year on this native grass.

Two species of *Euchlaena* have previously been reported as susceptible to infection with *Aplanobacter stewarti,* but neither is native to the United States.[3, 4]—CHARLOTTE ELLIOTT and ALICE L. ROBERT, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Geographical Distribution of Yellow Spot of Pineapples.[1]—In November, 1937, the writer visited the pineapple area centering in the town of Bathurst, Union of South Africa. The area is primarily devoted to the growing of the Queen variety of pineapple, but there are scattered Cayenne plantings throughout. Plants and fruits affected by symptoms indistinguishable from those of yellow spot of pineapples in Hawaii were found on both varieties. Infections were rare among young plants and on the crowns of fruits; but symptoms in the fruit were encountered frequently enough to indicate that the problem there was of some economic importance. One grower estimated that during the season just past he had discarded in the neighborhood of 10 per cent of the fruit prior to its shipment to the fresh-fruit market.

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[1] Hitchcock, A. S. Manual of the grasses of the United States. U. S. Dept. Agr. Misc. Publ. 200, 1040 pp. 1935.

[2] Mangelsdorf, P. C. and R. G. Reeves. Hybridization of maize, Tripsacum, and Euchlaena. Jour. Hered. 22: 329–343. 1931.

[3] Elliott, Charlotte and F. W. Poos. Seasonal development, insect vectors, and host range of bacterial wilt of sweet corn. Jour. Agr. Res. [U. S.] (In manuscript.)

[4] Elliott, Charlotte. Dissemination of bacterial wilt of corn. Iowa State Coll. Jour. Sci. 9: 461–480. 1935.

[1] Published with the approval of the Director as Technical Paper No. 99 of the Pineapple Experiment Station, University of Hawaii.

The relatively high incidence of the disease in fruits when compared with the very low incidence on leaves and crowns indicates that the vector in that area is primarily a flower feeder, and, in this connection, the possible relationship between the vectors of the *Kromnek* disease of tobacco and tomato and yellow spot of pineapple, in South Africa, is of great interest (Fig. 1).

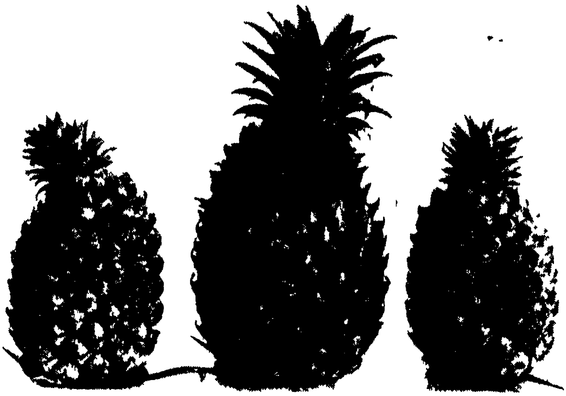

FIG. 1. Yellow spot disease from the Bathurst area, Union of South Africa. Note typical cavities in the fruit

Just prior to visiting the pineapple area the writer had the privilege of seeing the research on *Kromnek* disease at the private laboratory of E. S. Moore in the Kat River Valley and also the Department of Agriculture's laboratory at Fort Beaufort, where E. E. Anderssen was working on the insect vectors. Mr. Anderssen accompanied the writer into the pineapple area and was able to find ***Frankliniella schultzei*** (Trybom), an important vector of *Kromnek* disease, in the flowers of pineapple. In addition, *Kromnek*-diseased weeds were commonly scattered throughout the pineapple area. The relationship between *Frankliniella schultzei* and pineapple yellow spot would, therefore, seem to be well worth investigating.

A further significant note in this connection is the fact that Dr. Moore has recently obtained evidence indicating the co-identity of *Kromnek* disease with tomato spotted wilt.[2]

[2] Oral communication.

In May, 1938, the writer visited the commercial pineapple area on the Island of Mindanao, Philippine Islands, and to a lesser extent, on the Islands of Luzon and Cebu.

In view of Serrano's statement[3] that pineapple yellow spot is a serious disease on the Island of Mindanao, an intensive search was made for it in that area. This search was facilitated by the presence of a species of *Emilia* indistinguishable from the one that is the principal weed host of the virus in Hawaii.

The results of this search were entirely negative. No evidence of yellow spot was found either in young plants, fruits, or crowns of pineapple, or in *Emilia*, which was generally distributed throughout the plantations. There is, furthermore, no record of this disease ever having been seen in this plantation by the members of the staff there, although they are all familiar with it as it occurs in Hawaii.

Moreover, careful examination of a collection of 200 *Emilia* plants taken at random from pineapple fields failed to reveal a single *Thrips tabaci* or, for that matter, any other thrips species.

It is true that Serrano's drawings (Plates 1 and 4) depict typical yellow spot symptoms. It can be definitely stated, however, that his illustrations (Plates 2 and 3) are definitely *not* those of yellow spotted plants. The plants depicted in these illustrations are typical of those seen fairly frequently in the Bukidnon plantation, which are affected by an as yet undescribed disease with a symptom picture so different from that of yellow spot as to preclude the possibility that the two diseases are in any way related.—WALTER CARTER, Pineapple Experiment Station, University of Hawaii, Honolulu, Hawaii.

The Infection of Potato Roots by Heterodera schachtii.—Any method hitherto described for estimating the amount of eelworm disease in a crop of potatoes has been dependent on a count of the cysts on the roots, an extremely long and difficult task. During the course of an investigation on the control of *Heterodera schachtii*, the following method of directly counting the amount of infection gave consistently good results when compared with the resultant crop.

The method consisted essentially of taking representative samples of the roots of the plants in the field, then, by a suitable staining technic, to count the number of roots containing larvae and express this number as a percentage of the number of roots examined. The roots were collected in the field and stored in glass bottles of damp earth until they could be brought into the laboratory and washed clean. They were then transferred to 90 per cent alcohol, where they could be stored indefinitely.

In sampling these roots before staining, lengths of 0.5 inch were taken from the finer roots, care being taken that the rootcap was included. This latter precaution ensured that the roots under examination were of very nearly the same age and obviated the chance of a longer exposure to infection. These lengths were then stained in a 2 per cent solution of iodine in alcohol for 20–30 minutes, and then destained in 90 per cent alcohol until the roots were quite clear. The roots were then cleared in clove oil until all but the root tip was transparent, after which they were mounted in a drop of clove oil and examined.

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[3] Serrano, F. B. Pineapple yellow-spot in the Philippines. The Philippine Journal of Science. 58: 481–491. 1935.

On microscopic examination of the roots, the larvae could be seen stained a dark brown, as they are more retentive of the stain than the root itself. The number of rootlets containing larvae could be counted and, hence, the percentage infection obtained. In the investigation here reported it was found that the number of infected rootlets gave as good results as the number of larvae in the roots. This held good until the potatoes were about 4 months old, under Scots conditions, at which time the number of larvae was the only reliable index, as larvae could be found in all the rootlets examined.

It has been pointed out that the time of infection of the potato roots is a vital factor in the amount of damage done to the crop by the parasite, and using the above method one can follow the course of early infection in infected fields under experimental treatment. With the permission of Dr. D. G. O'Brien, under whose supervision this investigation has been carried on, I am allowed to publish the following table showing the amount of early root infection of potatoes by *Heterodera schachtii* compared with the yields obtained from the resultant crop. The results were obtained in a field showing eelworm infestation, from a series of plots in quadruplicate, using the randomised block method of distribution.

Percentage Infection	Yield in Tons/Acre
67	6.6
38	9.3
29	10.2
20	11.1

The infection is based on the average of 4 plots, each furnishing 20 rootlets, and the yield is also the average of the 4 plots.

A full account of the experiments with special reference to the control of the disease will be published later by this department.—ALAN R. GEMMELL, Plant Husbandry Dept., West of Scotland Agricultural College, Auchincruive, Ayr, Scotland.

Control of Powdery Mildews with a Water Spray.—The superficial existence of most powdery mildews on the surfaces of their hosts, their inability to regenerate themselves from haustoria, and the known injurious action of rain on certain powdery mildews[1] indicated the possibility of the control of these fungi with a water spray. While powdery mildews can generally be efficiently controlled with standard fungicides, the factors of host injury, fungicide deposit, and convenience are sometimes against the use of fungicides. These objections would be partly met if a successful water-spray treatment were available. The common opinion that powdery mildews, like many parasitic fungi, are favored by rain, high humidity, and free moisture probably has hindered experimental use of water as a control agent, though some greenhouse operators have observed that rose mildew was checked by the heavy syringing with water, commonly employed in the control of red spiders.

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[1] Yarwood, C. E. The tolerance of *Erisiphe polygoni* and certain other powdery mildews to low humidity. Phytopath. 26: 845–859. 1936.

Control tests with water were made with the following powdery mildews: Euonymus mildew (*Oidium euonymi-japonici* (*G. arcangeli*) Sacc.) on *Euonymous japonica* L.; rose mildew (*Sphaerotheca pannosa* (Wallr.) Lév.) on *Rosa* sp., variety Dorothy Perkins; bean mildew (*Erysiphe polygoni* DC.) on *Phaseolus vulgaris* L. variety Pinto; cucumber mildew (*Erysiphe cichoracearum* DC.) on *Cucumis sativus* L.; and barley mildew (*Erysiphe graminis* DC.) on *Hordeum vulgare* L.

Water at about 70 pounds' pressure was applied in the late afternoon with considerable force, to get a washing effect for a few seconds in each treatment. With *Euonymus* the water was directed by compressing the water at the hose orifice with the thumb; in treatments on other hosts the water was directed through an adjustable spray nozzle. Water was directed to both leaf surfaces; with cucumbers and beans each leaf was held in the hand and treated individually, in other cases the entire plant or group of plants was treated as a unit. On beans and cucumbers this severe method

TABLE 1.—*Control of powdery mildews with water spray*

Host	Number of units in each treatment	Period of treatment	Amount of infection	
			Control	Plants sprayed with water
Euonymous	3 plants	About once every 5 days, May 28 to Aug. 30	78[a]	33[a]
Rose	6 pots of one plant each	Every 3 days for 4 weeks, starting 2 weeks after inoculation	72[b]	21[b]
Cucumber	2 pots of one plant each	Once at 3 days, and once at 6 days after inoculation, examined at 11 days	615[c]	0[c]
Bean	2 pots of two plants each	Every day for 11 days, starting at 24 hours after inoculation	1600[c]	20[c]
Bean	2 pots of two plants each	Once at 3 days and once at 6 days after inoculation, examined at 11 days	1600[c, d]	381[c]
Barley	5 pots of about 15 plants each	Once at 9 days and once at 11 days after inoculation, examined at 15 days	1228[e]	42[e]

[a] Percentage leaves infected, 200 counted in each treatment.
[b] Percentage leaves infected, 1400 counted in each treatment.
[c] Total number of colonies on 2 plants.
[d] Same control as above.
[e] Total number of colonies on 4 typical plants.

of treatment quickly caused watersoaking of the leaves, with subsequent injury, if the spray was directed to the lower side of the leaf for more than a few seconds; but such injury was of less importance with *Euonymus,* rose, and barley.

Heavily infected plant material was paired for comparability before treatment, and one member of each pair was retained untreated as a control. With *Euonymus,* naturally infected outdoor hedge plants were used; in other cases the plants were inoculated artificially. Data in frequency of treatment and results are given in table 1. In all cases, including other repetitions not tabulated here, marked mildew control was secured.

In the case of *Euonymus* and rose, especially, the tabulated data do not adequately indicate the differences between treated and control plants, since the infected leaves of the treated plants were much less severely infected than those of the control plants. With roses, marked control of red spider (*Tetranychus telarius* L.) was also secured with the water spray. On control plants 13 per cent of the leaves showed symptoms of red-spider injury, while on treated plants less than 1 per cent of the leaves showed such symptoms.—C. E. YARWOOD, Division of Plant Pathology, University of California, Berkeley, California.

Isolation of Phytophthora spp.—Trouble is sometimes experienced in the isolation of *Phytophthora citrophthora* and *P. parasitica* from citrus gummosis cankers, specimens of affected twigs, and diseased nursery stock because of the difficulty of ridding the plantings of bacteria and other microorganisms. Pure cultures of the fungi are readily obtained by use of the following procedure. The diseased bark is freed from dirt by a thorough scrubbing in running water. The specimen is then supported on hardware cloth at the top of a glass container, so that the diseased portion is touched by the water but is not completely immersed. After 3 or more days in the running water, depending mainly on its temperature, mycelium of *Phytophthora* spp. and other fungi have grown out from the bark, and, after 5 days to a week, the former usually have produced sporangia. Several freshly picked, turgid lemon fruits in the silver or early tree-ripe stages are placed on the hardware cloth with the diseased specimen. The sporangia are then induced to form zoospores[1] by replacing the tap water with water cooled to 15° C. The zoospores readily invade the lemon rind and typical brown rot infections appear in 4 to 7 days. The fruit is then removed and the decay allowed to progress for several days to a week, or until the lemon is thoroughly invaded by the *Phytophthora* hyphae, but before invasion by secondary *Penicillium* spp. The surface of the lemon is then flame-sterilized, the fruit is cut open, and its seeds removed aseptically to glucose-potato-agar or other suitable medium. From active cases of gummosis pure cultures of these species have been invariably recovered by this method. It may also be used to free cultures of *Phytophthora* spp. from contaminating bacteria and fungi.[2]

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[1] Fawcett, H. S., and L. J. Klotz. A procedure for inducing the production of the sporangial and swarm stages in certain species of Phytophthora. Phytopath. 24: 693–694. 1934.

The technique, with some modifications, may be found of use in the isolation of other species of *Phytophthora* and other plant pathogens that form swarm spores.—L. J. Klotz and H. S. Fawcett, Citrus Experiment Station, Riverside, California.

Downy Mildew of Tobacco in Brazil.—Approximately a year ago Mr. J. C. Hart told me that downy mildew had for two seasons occasioned serious losses of tobacco seedlings in the State of Rio Grande do Sul, Brazil. To students of tobacco diseases this observation is of particular interest, especially as regards the identity of this downy mildew. It may be recalled that Spegazzini, in 1888, first collected *Peronospora nicotianae* at Buenos Aires, Argentina, a location not far distant from Rio Grande do Sul. In September, 1938, Mr. Hart gathered a small quantity of diseased tobacco leaves, placed them for 24 hours in lactic acid to kill the pathogen and to clear the tissues, and then sent them to me for examination. Sporangiophores, characteristic of *Peronospora*, were found to be abundantly present on the lower surface of these leaves. Within the leaf tissues, moreover, glabrous oospores, indistinguishable in appearance from those of *Peronospora tabacina,* also were found in abundance. It must be concluded, therefore, that two species of *Peronospora* occur on *Nicotiana* in South America. The one, *P. nicotianae,* has long been present, but it is highly probable that *P. tabacina* was only recently introduced. Of course, the manner of its introduction into Brazil, just as into the United States, remains unknown.—F. A. Wolf, Duke University, Durham, North Carolina.

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[2] Fawcett, H. S. Gummosis of Citrus. Jour. Agr. Res. [U. S.] **24**: 191–236. 1923.

ADVICE TO AUTHORS

If you've got a thought that's happy—
Boil it down.
Make it short and crisp, and snappy—
Boil it down.
When your brain its coin has minted,
Down the page your pen has sprinted,
If you want your effort printed,
Boil it down.

Take out every surplus letter—
Boil it down.
Fewer syllables the better—
Boil it down.
Make your meaning plain—express it
So we'll know—not merely guess it,
Then, my friend, ere you address it,
Boil it down.

Skim it well—then skim the skimmings—
Boil it down.
When you're sure 'twould be a sin to
Cut another sentence in two,
Send it on, and we'll begin to
Boil it down.

(From *Radiography and Clinical Photography*, June 1938—Vol. 14, No. 3)

FRED CAMPBELL MEIER
1893–1938

ROYAL J. HASKELL AND HOWARD P. BARSS

As Pan American Airways' giant Hawaii Clipper sped westward toward Manila on Friday, July 29, 1938, it carried Fred Campbell Meier, plant pathologist, and Earl B. McKinley, his medical comrade, bound on what they expected to be the first of a series of scientific biological explorations of the earth's atmosphere. Out into space went the radio messages:

> "4.03 a.m. Greenwich Central Time.[1] Temperature 13° C. Altitude 9100 ft. Wind 19 knots with rough air. Latitude 12° 27′ N. Longitude 130° 40′ E. Ground speed 112. Desired track 282°. Weather—rain. Ten strato cumulus at 9200 ft. Ten cumulus at 9200 ft. Radio bearing 101."
>
> "Stand by for one minute as I am having trouble with rain static."

Such were the last words of any kind from the transport. One of the greatest searches ever conducted on the Pacific revealed no clue to the fate of the airship. Samples of the oil found near the Clipper's course by the Army transport Meigs about 600 miles southeast of Manila proved to be unlike the oil used by the lost craft.

Whatever happened to the "Hawaii Clipper" and those on board, Meier, McKinley, the other four passengers, and the nine members of the crew, may always remain another of the hidden mysteries of the sea. If there is any consolation for Meier's friends it is in the fact that this adventurous pioneer met his end in the way he would have it—in performance of duty, in devotion to science.

Fred had left Washington in high spirits. The opportunity to devote himself to the work in which he was most deeply interested was at hand. He was getting back into plant pathology. His dreams of the development of a far-reaching field of science, which he designated "aerobiology," were beginning to be realized. He had been authorized by the United States Department of Agriculture to initiate a special research project in cooperation with the National Research Council. At the start the work was to be a study of organisms in the air with special reference to plant pathogens and wind-blown pollens. Since large bodies of water offer opportunity to measure distances over which spores and pollen grains may be carried by winds, he thought it best to begin by sampling air masses over the Pacific Ocean and to train flight personnel of Pan American Airways to make regular air collections for him.

Accordingly, plans were made for departure from California. Accompanying him was Dr. Earl B. McKinley, Dean of the School of Medicine, The

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[1] 12:03 p.m., Friday, July 29, Manila Time; 11:03 p.m., Thursday, July 28, Eastern Standard Time.

George Washington University, Washington, D. C., a friend and enthusiastic fellow member of the National Research Council Committee, which was guiding this project. He was to aid Meier with his collections as far as Manila and there would conduct experiments relating to leprosy. Meier intended to continue on to Hong Kong, come back on the same plane and pick up McKinley on the return flight. On July 23 the two scientists winged their way westward out through Golden Gate on the journey that was never completed.

Fred Campbell Meier was born April 5, 1893, at Riggston, Illinois, where his father, W. H. D. Meier, was a rural school teacher, later superintendent of schools in other towns in that State. This remarkable man, the elder Meier, already a devoted biologist, brought his family, in 1909, from Illinois to Cambridge, Massachusetts. Here, and later at Framingham, Massachusetts, he taught biology; but, on the side, he pursued advanced studies at Harvard University, which granted him, at the age of 51, the degree of Ph.D. in that subject. A descendant of German farmers, he broke away from the ancestral tradition and gave himself and his family to the advancement of science. Upon his children, in large measure, he bestowed his own mental vigor, enthusiasm, and persistence. His three daughters, Lois, Florence, and Dorothy, as well as his son, Fred, also have won distinction in the field of biology.

The father and mother continue to reside at Framingham. Dr. Lois Meier Shoemaker is Assistant Professor of Science, State Teachers College, Trenton, New Jersey; Dr. Florence E. Meier is with the Smithsonian Institution at Washington, D. C.; and Dr. Dorothy Meier is teaching at Hunter College, New York City.

Fred's wife, Agnes Eastman Meier, formerly of Framingham, Massachusetts, whom he married in 1920, continues to reside at the home in Chevy Chase, Maryland. This devoted companion was a constant source of help and encouragement. She worked with him and at times went out into the field with him, assisting in making observations, taking notes, and editing reports. She relieved him of much of the secretarial and business management load connected with PHYTOPATHOLOGY and the Society. How could he have carried on his many activities and performed the valuable services that he did without her active and helpful cooperation?

Entering high school in Havana, Illinois, Fred continued for two years at the Rindge Manual Training School, Cambridge, Massachusetts, and finished at the Framingham High School. Working on farms during some of his high-school and college vacations gave him useful agricultural experience.

Entering upon a general scientific course at Harvard in 1912, Meier received his S.B. degree in 1916 and his A.M. in 1917, majoring in Cryptogamic Botany under Dr. Roland Thaxter, whom he assisted in the laboratory, and holding an Austin Teaching Fellowship for nearly two years. As an undergraduate, he assisted Dr. W. J. V. Osterhout in course work in elementary botany. Upon completion of his master's degree, he continued work at Harvard for the doctorate, but this was interrupted almost at once by the duties of his appointment in the United States Department of Agriculture. He persisted, however, in devoting what brief periods he could get in the succeeding years to continued graduate work. In this way, at the time of the ill-fated flight, all doctor's requirements had been met except for the completion of his dissertation on studies in aerobiology under Dr. W. H. Weston, Jr. His extensive collections of air-borne fungi and his bibliography and abstracts on aerobiology have been placed in charge of the Division of Mycology and Disease Survey, United States Bureau of Plant Industry.

After his junior year in college Meier obtained a summer appointment for laboratory and field work on truck-crop diseases in the Department of Agriculture at Washington under Dr. W. A. Orton, Office of Cotton and Truck Disease Investigations. Fond of outdoor life, he decided one day to take a well-earned afternoon off and go fishing down the Potomac. On the way he passed freight yards where he noticed piles of spoiled watermelons rotting along the tracks. A good look convinced him that this was the work of some plant disease. He immediately called off the fishing trip and took samples of the decayed melons back to the laboratory and made isolations. Buying several sound melons with his own hard-earned cash, he later inoculated them with the fungus he had isolated. To his delight they came down with clear-cut cases of Diplodia rot. This new knowledge was the start of his interest in watermelon diseases and the subject of his first paper, "Watermelon Stem End Rot," published in the *Journal of Agricultural Research* in 1916.

For two more seasons he carried on summer work under Orton, conducting surveys, studies, and field experiments, in Florida and Georgia, particularly, on the control of anthracnose and other diseases of watermelon. Beginning in 1918 he continued this work on a full-time basis, as assistant pathologist. He demonstrated that anthracnose damage could be largely prevented by spraying, showed that disinfection of melon seed is of value, and proved that Diplodia infects melons through the freshly cut stems and develops in transit. He then devised and helped chemical companies perfect the Bordeaux-paste stem-end treatment of melons at the time of shipment. This became generally adopted and cut down to almost nothing the losses that once ran as high as 30 to 50 per cent in a carload. It was estimated that in 1920, because of this treatment, something like $121,000 was added to the incomes of Florida growers. Such success was attributable not only to Meier's well-conducted research work but also, in large degree, to the successful extension campaign that he waged through illustrated lectures, meetings, demonstrations, posters, and work with railway agents, dealers, county agents, and growers, in which he demonstrated everywhere his happy faculty for making friends, arousing enthusiasm, gaining support, and creating a generally favorable attitude on the part of others toward his work.

Turning his attention from melon-disease work to the relatively new and unexplored field of market pathology, from headquarters in New York City, Meier directed the Government investigations on diseases of fruits and vegetables in the markets from Buffalo and Pittsburgh eastward, from August, 1919, through June, 1921. He trained food-products inspectors in disease identification, advised on control practices, helped prepare the series of colored illustrations for the inspectors' market-disease handbook, and worked out the details of the market-inspection certificates and methods of analyzing the data, gave many illustrated lectures before important organizations, prepared, with Link, six Department of Agriculture circulars, and constantly shaped up the research program better to meet market needs in the light of new problems as they appeared. During this period he developed a wide circle of friends among leaders in railroad, distributing, and producing groups.

A third important phase of Meier's work was entered into when, in July, 1922, as the first regular Federal extension pathologist, he began serving as connecting link between the plant pathologists of the Government and the State extension services, which were beginning to employ plant-disease specialists, acting as adviser and collecting and disseminating helpful information on effective control practices and extension methods. This work took him to all parts of the United States and broadened his acquaintance with staff members in the land-grant colleges, with county agents, with farmers, and with key men in many commercial, civic, and trade groups. He helped everywhere in planning and in conducting meetings, demonstrations, and surveys. At his Washington headquarters, as time permitted, he helped prepare popular plant-disease publications and inaugurated the mimeographed "Extension Pathologist." He aided in bringing workers together for needed conferences, like the one held in Washington in 1929 on Tobacco Diseases and Nutrition Problems. He also organized extension discussions at the annual meetings of The American Phytopathological Society.

Outstanding was the success with which Meier enlisted the help of extension specialists, county agents, members of the grain trade, chemical manufacturers, and others in a nation-wide wheat-smut campaign, which led to the extensive use of seed treatment with copper carbonate dust. In this connection he started the analysis of wheat terminal-inspection reports, which showed the need for seed treatment of wheat and later proved its effectiveness.

Concerning his work as extension pathologist, A. B. Graham, of the Extension Service, under whose guidance Meier worked, writes:

> "He possessed a happy combination of the research and extension mind; he desired to be sure of his facts, he was tactful in his approaches to fellow workers in the Department and in the States—yielding on matters not of greatest importance but everlastingly holding to those he considered fundamental and vital. He had unlimited patience and an unconquerable faith in the dominance of right.
>
> "His everyday dealings with those in his field of work paved the way for a general acceptance of his methods of extending the work in plant pathology."

From February 1, 1930, to July 23, 1934, as Principal Pathologist, in charge, Meier successfully administered the work of the Federal Division of Barberry Eradication, directing 12 State field offices, each with a large personnel, and supervising research designed to direct the course taken in its program. In this position his planning and organizing ability and value as an administrator were well shown. He stressed the educational phases in which he enlisted the Extension Service and the public-school systems. He adopted the policies of giving the field work, wherever possible, to local labor and of using work-relief funds for barberry eradication.

Then, back to the Extension Service he went, this time as Federal leader of county agricultural extension work in the 12 Eastern and Northeastern States. Here, he advised State extension leaders, county agricultural agents, and specialists in developing programs and making their work more effective. He also helped in correlating the large national programs for agriculture with extension work. Of these phases of his work his associate and immediate supervisor, H. W. Hochbaum, says:

> "Fred Meier was loyalty personified, loyal to the work, loyal to his associates, loyal to the workers in the States. His help was invaluable. No job was too hard, no day was long enough. His great range of interests and information and his many contacts made his work most effective and along with his kindness, considerateness and tact made him hosts of friends."

His fellow plant pathologists in all parts of the country testify, in addition, to his complete professional unselfishness and his lifelong devotion to helping others at every opportunity.

Meier joined The American Phytopathological Society in 1919, became a sustaining life member, and served the Society actively and effectively in important posts. He rendered valuable assistance on various committees. From 1925 to 1927, inclusive, he was Secretary of the Advisory Board. He ably served the Society as Secretary-Treasurer and Business Manager of PHYTOPATHOLOGY for six years, 1929–1934, inclusive, and as Vice President, in 1935. He was a Fellow of the American Association for the Advancement of Science and a member of the Botanical Society of Washington, and of the Cosmos Club, and the Torch Club, of Washington, D. C.

At the time the concepts were being formulated that led to the organization of the Tropical Plant Research Foundation, which began operations in 1924, Meier and his wife, along with W. A. Orton and G. R. Lyman, were active prime movers of the enterprise. Although it was brought to a state of inactivity by the world-wide depression, when business recovery seemed in sight, Meier again went quietly to work toward its rejuvenation as a bond of union in agricultural progress for Pan American nations.

On July 9, 1938, Meier was given what was intended to be a temporary transfer to the Bureau of Plant Industry to initiate a study of the aerial dissemination of plant pathogens and plant pollens. Since high-school days he had been interested in airplanes, and, later, in their use in the dusting of plants for insect and disease control. In 1930 he became actively interested in the use of airplanes to determine the distribution of the spores of fungi in upper-air currents. The barberry-eradication and grain-rust-control program of the Department of Agriculture brought him in close contact with Stakman and his associates who, as early as 1921, had used airplanes to collect rust spores at various altitudes. The need for and valuable possibilities that lay in such studies appealed strongly to Meier. From 1930 on he devoted almost all his spare time and energy and drew upon his private resources to advance what became the major scientific interest of his life. He believed that the air would yield a vast amount of useful information if one could only devise effective ways of collecting and interpreting air-borne material.

With the cooperation of air-service units of the Army, Navy, and Coast Guard, and commercial air transport lines, the Department of Agriculture arranged for Meier to make aerial collections of microscopic organisms from time to time during routine flights over various sections of the United States. He continued his studies during the training flights of the U. S. Dirigible Los Angeles in the winter of 1931, devising a technique for making living collections that could be returned to the laboratory and grown in artificial cultures for further study. He continued to fly whenever the chance presented itself. The Bureau of Plant Industry assisted Meier with laboratory facilities for the culture of the fungi collected on these flights. Later, he assisted in a study of pollen distribution over the seed-beet fields of the Mesilla Valley in New Mexico.

During their 1933 North Atlantic-Greenland flight, Col. and Mrs. Charles A. Lindbergh made aerial collections for Meier, using the famous Lindbergh-Meier "sky-hook" equipment, which, in improved form, has become standard for such investigations. Meier also arranged for collections during the 1934 flight of the U. S. Army planes to Alaska and return. With the aid of the Pan American Airways, he, himself, made valuable collections over the Caribbean Sea. In 1935, collections of great interest were made at the instance of Meier during Major Albert W. Stevens' record-breaking National Geographic–U. S. Army stratosphere flight. At that time, living spores of ten different fungi were obtained above 36,000 feet. Amelia Earhart, during her ill-fated world flight in 1937, in a telephone conversation from Java with her husband, reported making, for Meier, systematic aerial collections and notes. These were all lost with her.

After tireless efforts by Meier to arouse more active general interest in such study, the National Research Council became attracted to the possibilities of a more intensive exploration of the content of the air with particular reference to the microscopic agents that cause disease of plants, animals, and humans, including pollens that produce hay fever. At a conference held in Philadelphia, January 23, 1937, it was suggested that an interdivisional project on the aerial dissemination of pathogenic organisms be started. At a meeting of the National Research Council's Interdivisional

Committee on Border Land Problems in the Life Sciences in Washington on April 25, 1937, following a presentation by Meier of the preliminary efforts that had been made to collect microorganisms from the air, the chairman of the Division of Biology and Agriculture was asked to form a committee to deal with this problem. This committee was promptly organized and Meier was asked to assume the chairmanship. Men of distinction from the fields of medical science, public health, aeronautics, engineering, meteorology, agricultural research, bacteriology, plant pathology, and botany, as well as representatives of the Army and Navy and the heads of three divisions of the National Research Council, were included. Among these was his friend McKinley.

This committee, later known as the Committee on Aerobiology, held its first meeting on November 12, 1937, in Washington. Plans for the future were launched enthusiastically. During the following winter much foundation work was done by Meier and his committee to outline possibilities and to find ways and means of getting a constructive program started. In the spring of 1938 the U. S. Department of Agriculture cooperated by setting up a special research project on aerobiology. This project was to have been active during July and August of 1938 and the spring months of 1939, with Meier in charge, the Department of Agriculture having generously arranged for temporary leave of absence from the Extension Service so that he might inaugurate this important work in aerobiology. It was on this project that Meier was engaged during his last flight.

Meier's persuasive enthusiasm brought much support for the work of his committee. The Carnegie Corporation of New York offered invaluable financial aid for the exploratory period. Air services and individuals offered helpful support, not only with the thought that the immediate project would yield results significant to the advancement of science and the promotion of human welfare but also with the hope and confidence that the project would prepare the way for continuing activity in the development of aerobiology with all its scientific and practical implications.

Dr. R. E. Coker, Chairman of the Division of Biology and Agriculture of the National Research Council, has paid tribute to Meier in the following words:

> "Almost exclusively by his own personal devotion to the cause, by his scientific vision of the possibilities, by his extraordinary physical and mental energy, and by his commanding enthusiasm, unmistakable sincerity and personal persuasiveness, he had, while giving full time to duties quite unrelated to the study of air content, enlisted the active interest and aid of Government and commercial air services and of technicians and scientists in the development of apparatus for collections, and in the accumulation and identification of material. . . . Just before his tragic end the stage seemed well set for the early realization of some of his scientific dreams and the practical fruition of his long continued effort to establish a comprehensive and effective project in the study of aerobiology."

PLATE I

1. F. C. Meier (left) and E. B. McKinley (right) examining improved Lindbergh-Meier "sky hook" immediately before departure from Washington, D. C. July 19, 1938. 2. Making a test of the equipment used in the National Geographic Society–Army Air Corps stratosphere flight of 1935. 3. Meier, in cockpit of open, two-seated, U. S. Coast Guard biplane, demonstrating early method of exposing culture dish. Cape May, N. J., 1931.

Meier's all-absorbing interest in science was in its relation to human welfare. The loss to the world of such a martyr to scientific progress is immeasurable. At the age of forty-five, after ten years of preliminary air-scouting to which, as an avocation, he had given without stint his vacations and out-of-office hours, Meier was hopefully starting a major organized exploration to extend scientific knowledge along new and important frontiers. To this field of aerobiological research; with the aid of others whom he had inspired, he might well have devoted the remainder of a long and active life. It will be impossible to replace a man who combined such courage, keenness of mind, breadth of outlook, and organizing ability with such an unfailing spirit of consideration and friendliness.

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RELATION OF PARTICLE SIZE AND COLOR TO FUNGICIDAL AND PROTECTIVE VALUE OF CUPROUS OXIDES[1]

JOHN W. HEUBERGER AND JAMES G. HORSFALL[2]

(Accepted for publication February 15, 1939)

INTRODUCTION

In any collection of cuprous oxides the number of colors is almost as large as the number of samples. The colors range from red through orange and yellow to green. The presence of varying amounts of black cupric oxide in any of these imparts various shades of brown.

The early research on red cuprous oxide indicated that the seed protective value diminished as the color darkened with oxidation to cupric oxide (4). It was found later (6), and confirmed by Anderson *et al.* (1), that cupric oxide is inferior to cuprous oxide as a fungicide. Recently, cuprous oxide powders of varying colors have been introduced as fungicides. Preliminary microscopic examination of several of these powders confirmed what Weiser (17) had pointed out, that the particle size decreased as the color shifted toward yellow. This observation led to the present investigation of the relation of particle size and color to fungicidal and protective value.

MATERIALS AND METHODS

Commercial cuprous oxides may be prepared by 3 general methods: precipitation by reducing agents, by electrolysis, and by heating metallic copper and cupric oxide together in the absence of air, or by pulverizing the scale produced in rolling sheet copper. Only thermal[3] and electrolytic oxides were used in the experiments.

A large series of cuprous oxides available in the market was assembled. This series included thermal cuprous oxides (prefix T), electrolytic red oxides (prefix R), and electrolytic yellow oxides (prefix Y). To this group was added a series of experimental samples prepared especially for this project (Table 1). As can be seen from the table, these oxides varied in color according to Ridgway (12) from red-brown through red and orange to yellow. The approximate wave length of reflected light was obtained by interpolation on Ridgway's chart (12). All samples were received as powders.

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[1] Approved by the Director of the New York State Agricultural Experiment Station for publication as Journal Paper No. 314, Feb. 8, 1939. Part of a research project conducted in cooperation with The Crop Protection Institute.

[2] The writers express their thanks to several manufacturers for the provision of experimental materials. Metals Refining Co., Hammond, Ind., and the Nichols Copper Co., New York, N. Y., supplied the thermal cuprous oxides. Mallinckrodt Chem. Co., St. Louis, Mo., Merck & Co., Rahway, N. J., and Röhm & Haas Co., Inc., Philadelphia, Pa., provided the electrolytic red cuprous oxides. Dr. L. C. Hurd of the last-named company has generously prepared for this project several samples, especially the yellow cuprous oxide.

[3] Thermal cuprous oxides can always be distinguished by a characteristic red-brown color. According to Ridgway (12) this red-brown is made up of red color plus a percentage of black. To the person not familiar with color values, this red-brown appears to be purple and it has been so published (3, 5).

Editor's note. Cost of publication of this paper was defrayed by The Crop Protection Institute.

TABLE 1.—*Samples of cuprous oxide under experimental test*

Legend	Method of manufacture	Mean particle diameter	Color	Wave length
		μ		Å
T 1	Thermal	2.93	Dark livid brown	6440
R 1	Electrolytic (partly oxidized)		Chocolate	6440
R 2	Electrolytic	1.65	Orange Pompeian red	6300
R 3	Electrolytic	1.95	Pompeian red	6330
R 4	Electrolytic	1.94	Pompeian red	6330
R 5	Electrolytic		Pompeian red	6330
R 6	Electrolytic	2.57	Acajou red	6440
R 7	Electrolytic, R 6 (mill-pulverized)	1.87	Pompeian red	6330
R 8	Electrolytic, R 6 (air-pulverized)	1.47	Orange Pompeian red	6300
R 9	Electrolytic		Pompeian red	6330
Y 1	Electrolytic	0.94	Mars yellow	5950

Technique for Measuring Particle Size

Particle size is a telescoped expression referring here to the average diameter of the individuals in a particle-size population of a powder. More particularly it is the diameter of the particle of average weight. The particles in most fungicides tend to clump or aggregate. When the mean diameter refers to both discrete particles and clumps it is referred to as "effective particle size." This expression is best used in referring to particles of dusts, where the aggregates are not deliquesced as they tend to be in spray water.

The technique for measuring particle size was a modification of that proposed by Wilcoxon and McCallan (18) as "a fairly rapid and simple method that permits calculation of an average particle size, namely, the diameter of a particle of average weight, (which) can be based on microscopic counts of the number of particles lying within a known area . . . if the weight applied to the area is known."

The method was adapted to spray suspensions, so that whatever aggregation occurred in the preparation of the suspension would be reflected in the results. The suspensions were prepared in the usual way by pasting the materials in a little water in a mortar before diluting to spray concentration. After thorough shaking, a subsample was pipetted rapidly to a Levy blood-counting slide, where the volume of liquid under the cover slip is known.

After allowing 5 minutes for the particles to settle, the slide was placed on the stage of the microprojector described by Streeter and Rankin (13). This projector was fixed permanently in a photographic dark room to give a magnification of 1900 diameters on a screen ruled into 100 10-μ squares. The number of particles on each square was then determined. To reduce sampling error 1 field on each of 5 slides was examined to give 500 squares.

The average number of particles per square was then calculated. The weight of particles per square was calculated from the concentration of material in the liquid and the volume of liquid for each square ($10\,\mu \times 10\,\mu \times 200\,\mu$). The particle size was calculated from the following formula:

$$\text{Mean particle diameter } \mu = \sqrt[3]{\frac{10^9 \times 6\,W}{\pi n p}}$$

where W = weight of material in milligrams per square, n = number of particles per square, p = density of material (cuprous oxide = 6.0).

For the special case of cuprous oxide the 6 in the denominator cancels the 6 in the numerator. Most work was done with 0.1 per cent copper = 236×10^{-10} mg. per square. Multiplying this by 10^9 leaves 23.6 in the numerator to be divided by 3.1416, leaving 7.512 in the numerator. In routine calculations this figure was divided by the average number of particles per square and the cube root was taken from a table to give mean particle diameter in μ. Other concentrations were made up in multiples of 0.1 per cent copper, so that 7.512 simply required conversion by the necessary factor.

If other copper materials are to be measured the density needs to be considered.

Wilcoxon and McCallan (18) have discovered a short cut in determining the number of particles per square. They make use of the fact that the sulphur particles they worked with follow the so-called Poisson distribution. In that case it is only necessary to count the number of squares in the 500 that contain no particles. The percentage (P) of these is calculated. The average number of particles can then be obtained directly from the graphs published by Thorndike (14).

Since the distribution of the cuprous oxide particles in the Levy cell also seems to follow the Poisson distribution, Thorndike's chart has been used to save considerable time. The summarized procedure is as follows:

1. Prepare suspension of Cu_2O with 0.1 per cent copper.
2. Pipette 5 samples to Levy counting slides.
3. Examine 100 ten-micron squares from each sample.
4. Ascertain the number of squares containing no particles.
5. Calculate average percentage of blank squares.
6. Obtain average number of particles per square from Thorndike.
7. Solve following equation

$$\text{Mean particle diameter } \mu = \sqrt[3]{\frac{7.512}{\text{no. particles}}}$$

Technique for Measuring Fungicidal Value

Since fungicidal value (6) is considered to be the ability of a fungicide to kill or inhibit a fungus, it can be measured in the laboratory in the absence of the plant and the complications of retention and tenacity (2). The technique for measuring fungicidal value was a modification (6) of that used by McCallan and Wilcoxon (10). Glass microscope slides, coated with cellulose nitrate, were sprayed for different exposure periods by placing a sliding shutter between the spray nozzle and the slide. In this way the slides received varying quantities of copper. The nozzle emitted 10 cc. of spray fluid in 36 seconds and applied 0.384 mg. of spray fluid per square centimeter per second on a slide 20 inches away. Using 0.1 per cent copper, the deposition of toxicant (copper) was 0.000384 mg. per square centimeter per second. The calculated copper concentration in the spray fluids was checked iodometrically at intervals.

During spraying, the suspensions were stirred mechanically to prevent sedimentation. The test fungus was *Macrosporium sarcinaeforme* Cav., approximately 21 days old, growing on potato-dextrose agar at 21° C. The spores germinated about 97 per cent from day to day in distilled water without the addition of stimulating substances.

The spore suspensions were made up in distilled water with 50,000 spores per cc. One drop (0.05 cc) of this spore suspension was placed on 3 different regions of the dry spray film. The drop on cellulose nitrate spread 7.5 mm. in diameter, which gave an average of 5,700 spores per square centimeter. The slides were placed in an inverted moist chamber, provided with a water seal, and incubated overnight at 25° C. One hundred spores in each of the 3 drops on duplicate slides were counted and the number of germinated spores was recorded.

In accordance with a suggestion from the current committee of The American Phytopathological Society on standardizing fungicide-testing technique, readings are expressed as percentages of spores not germinated to make them cognate with other toxicological tests. Two materials should be compared at the LD50 point (15), *i.e.*, the quantity of toxicant inhibiting 50 per cent of the spores (lethal dose 50 per cent). The LD50 point can be obtained by interpolation on the fungicidal value curve.

Technique for Measuring Protective Value

Protective value (the ability of a material to prevent infection of plant tissue) of the various cuprous oxides was tested on Perfection pea seeds and on rose and tomato foliage. The method of testing seed-protective value was as follows: Definite amounts of the various samples were weighed and added to 100 g. of pea seed in a 500 cc. Erlenmeyer flask. The flasks were placed on a revolving disk and rotated end-over-end for 10 minutes at 40 r.p.m. The seed was then planted in soils of high damping-off-inoculum potential (5) in the greenhouse, in the climate laboratory,[4] and in the field. The damping-off organism was chiefly *Pythium ultimum* Trow. At least four 25-seed replicates were used in the greenhouse and in the climate laboratory. Five 100-seed replicates were used in the field. Emergence counts were made after sufficient time had elapsed to allow for maximum emergence.

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[4] The climate laboratory was designed especially for research on pre-emergence damping-off and seed protection. It consists briefly of six Johnson chambers (Phytopath. 18: 227–238. 1928) placed in a refrigerated basement room. Each chamber is heated thermostatically. Each chamber is fitted with 4 series of 4 double-walled auto-irrigators giving accurate soil-moisture control. When seed protectants are being tested all chambers operate at the same temperature (usually 15° C.) and all irrigators at the same moisture content (usually 25 cm. water, capillary tension).

Foliage protective value was tested under field conditions with the usual spray methods. The materials were applied at equal copper concentrations.

EXPERIMENTAL DATA

The work on particle size and color of cuprous oxides was divided into 3 sections: (1) laboratory studies on their relation to fungicidal value; *(2) greenhouse, climate-laboratory, and field studies on their relation to seed-protective value; and (3) field studies on their relation to foliage-protective value.*

Fungicidal Value

Effect of Method of Manufacture. Cuprous oxides of various colors and manufactured in different ways (Table 1) were tested. In the first test the materials were all made electrolytically and the colors ranged from chocolate through various hues of red to yellow. The materials were compared at 0.2 per cent copper at a deposition of 0.01 mg. of copper per square centimeter (Table 2). It is clear from the data that, in general, fungicidal value increases as the color grades toward yellow.

TABLE 2.—*Fungicidal value of various samples of electrolytic cuprous oxides*

Material	Color	Mean particle diameter	Wave length	Spores not germinated[b]	Copper deposition
		μ	Å	*Per cent*	*mg./cm.*^{2} $\times 10^{-4}$
R 1[a]	Chocolate		6440	41.8	100
R 6	Acajou red	2.57	6440	53.0	100
R 9	Pompeian red		6330	64.2	100
R 4	Pompeian red	1.94	6330	81.0	100
R 2	Orange Pompeian red	1.65	6300	98.3	100
Y 1	Mars yellow	0.94	5950	100.0	50

[a] A sample partly oxidized to cupric oxide.

[b] Germination of non-treated spores was 99.0 per cent.

Two experiments were next made with 4 samples, 1 thermal, 2 electrolytic red, and 1 yellow, in which the particle sizes were known (Table 3). The data again show that the fungicidal value increases as the color shifts toward yellow, and that this increase is associated with a progressive reduction in particle size. On the basis of the LD50 values and on the basis of the quantity of copper per square centimeter that inhibits the same percentage of spores, it is interesting that the electrolytic red cuprous oxide is between 3 and 4 times as fungicidal as the dark, livid-brown cuprous oxide, and that the yellow oxide is about twice as fungicidal as the red and about 9 times as fungicidal as the dark, livid-brown sample. The order of magnitude for fungicidal value is about 1, 2, 8 for the red-brown, red, and yellow cuprous oxides.

Effect of Pulverization. If it is generally true that particle size, color, and fungicidal value are related, pulverization of a red sample should change the color toward yellow and should increase the fungicidal value.

TABLE 3.—*Effect of particle size and color on fungicidal value of cuprous oxides*

Material	Color	Mean particle diameter	Wave length	Experiment I[a]			Experiment II[a]		
				Copper deposition	Spores not germinated	LD50	Copper deposition	Spores not germinated	LD50
		μ	Å	*mg./cm.*^{2} $\times 10^{-4}$	*Per cent*	*mg./cm.*^{2} $\times 10^{-4}$	*mg./cm.*^{2} $\times 10^{-4}$	*Per cent*	*mg./cm.*^{2} $\times 10^{-4}$
Thermal oxide, T 1	Dark livid brown	2.93	6440	192 96 48	76.0 52.7 9.7	90	201 124 48	73.3 58.5 25.5	103
Electrolytic oxide, R 4	Pompeian red	1.94	6330	96 48 24	89.7 70.5 42.0	30	101 62 24	94.9 75.0 48.5	26
Electrolytic oxide, R 8	Orange Pompeian red	1.47	6300	96 48 24	96.0 73.0 28.0	30			
Yellow oxide, Y 1	Mars yellow	0.94	5950	48 24 12	100.0 85.0 54.7	9	50 31 12	100.0 95.5 55.5	11
Bordeaux				2	47.3	2[b]	3	60.0	2[b]

[a] Germination of non treated spores was 98.0 per cent in Experiment I and 98.2 per cent in Experiment II.
[b] Estimated.

Several experiments were made reducing the particle size of electrolytic oxide R 6 by pulverizing. A quantity of material was divided into 3 lots. One lot was kept as the original. The second lot was pulverized in a mechanical crusher to a finer particle size. The third lot was pulverized still further by an air-pulverizer known as a "Micronizer." The mean particle diameter of these 3 samples was determined. They were made up to 0.1 per cent copper and tested for fungicidal value. Data from a typical test are given in table 4.

TABLE 4.—*Effect on fungicidal value of reducing the particle size of electrolytic oxide R 6*

Sample no.	Method of preparation	Color	Mean particle dia.	Spraying time	Copper deposition	Spores not germinated[a]	LD50
			μ	*Sec.*	*mg./cm.*^{2} $\times 10^{-4}$	*Per cent*	*mg./cm.*^{2} $\times 10^{-4}$
R 6	Original	Acajou red	2.57	12.5	48.5	51.5	43.4
				7.5	29.0	41.7	
				2.5	9.7	14.8	
R 7	Mill-pulverized	Pompeian red	1.87	12.5	48.5	69.2	34.1
				7.5	29.0	44.2	
				2.5	9.7	9.7	
R 8	Air-pulverized	Orange Pompeian red	1.47	12.5	48.5	86.0	21.6
				7.5	29.0	67.8	
				2.5	9.7	22.7	

[a] Germination of non-treated spores was 98.0 per cent.

The color did shift toward yellow and the data show that the fungicidal value did increase as the particle size was reduced by pulverizing.

Effect of Fractionation. It was obvious from microscopic observation that the samples of cuprous oxide were composed of a population of different particle sizes. It was noted also in the preceding experiments that the water suspension of the thermal oxide on standing exhibited 2 distinct color fractions, a deep red-brown in the bottom of the flask and a yellow-brown above. The yellow-brown suggested the possibility that the sample contained some yellow cuprous oxide. To test this assumption, an experiment was made in which the sample was fractionated into several particle-size groups by elutriation (water flotation). The first fraction to settle from the water suspension was metallic-copper-colored and contained some metallic copper. The next was a deep brown, the next was red-brown, and the last was yellow-brown. Three particle-size groups, (a) metallic-copper-color fraction plus the deep brown fraction, (b) red-brown fraction, and (c) yellow-brown fraction, were made up to a liter in volumetric flasks and the copper content determined iodometrically. The following copper concentrations were then made and tested for fungicidal value: large particle-size group (a), 0.4 per cent copper; medium particle-size group (b), 0.4 per cent copper; small particle-size group (c), 0.02 per cent copper. These fractions were compared with the original sample at 0.4 per cent copper (Table 5).

TABLE 5.—*Fungicidal value of 3 particle-size groups in thermal oxide T 1 separated by elutriation*

Material	Particle-size group	Color	Copper deposition	Experiment I[a]		Experiment II[a]	
				Spores not germinated	LD50	Spores not germinated	LD50
			mg./cm.^{2} $\times 10^{-4}$	*Per cent*	*mg./cm.*^{2} $\times 10^{-4}$	*Per cent*	*mg./cm.*^{2} $\times 10^{-4}$
Thermal oxide, T 1	Original	Red-brown	201	86.0	104	64.0	138
			124	61.5		46.0	
			46	17.7		22.7	
	(a)	Deep-brown	201	72.7	123	59.5	147
			124	50.0		37.0	
			46	10.0		23.3	
	(b)	Red-brown	201	95.7	69	91.0	46
			124	87.3		83.0	
			46	34.5		49.7	
	(c)	Yellow-brown	10	47.7	9	61.3[b]	8
			6	24.0		42.3[b]	
			2	4.7		21.0[b]	

[a] Germination of non-treated spores was 97.5 for Exp. I and 98.0 for Exp. II.
[b] Copper concentration twice that in Exp. I.

The data, as expected, show that the thermal oxide sample T 1 was composed of particles of various sizes that varied widely in their fungicidal value. The large particle-size fraction (deep-brown-color) was lower in fungicidal value than the original sample, while the medium particle-size fraction (red-brown-color) was higher; the small particle-size fraction (yellow-brown-color) was extremely high in fungicidal value, being about 9 times as fungicidal as the original sample. It is of interest to recall here that the pure yellow cuprous oxide sample Y 1 was 9 times more fungicidal than the thermal oxide T 1 in the data presented in table 2. This means that the thermal cuprous oxide T 1 did carry a yellow fraction, as suspected, and that this yellow fraction was just as fungicidal as pure yellow cuprous oxide.

Since these data indicate that a large proportion of the fungicidal value of the thermal oxide T 1 may have been due to the small particle-size fraction, an effort was made to determine what proportion of the original sample was composed of this fraction. A 20-gram sample was separated by elutriation into 3 particle-size fractions based on the settling rates of the particles. The copper content of each fraction was then determined iodometrically. The data are as follows:

Large particle-size fraction	80.4 per cent
Medium particle-size fraction . . .	14.8 " "
Small particle-size fraction	4.8 " "

A sample of electrolytic oxide R 4 also was separated into 2 particle-size fractions by elutriation. The first fraction to settle was orange-red and comprised 87.5 per cent of the total, whereas the other fraction, the last to settle, was a pronounced yellow-red and comprised 12.5 per cent of the total. The orange-red fraction showed much less fungicidal value than the yellow-red.

Effect of Aggregation. Macroscopic and microscopic examination of various lots of cuprous oxide showed that some samples were more aggregated than others. This was reflected in a deeper color. Fungicidal value readings on aggregated and non-aggregated samples were made from time to time with similar results. Two tests illustrate the trends. In one test at 0.2 per cent copper, 31.5 per cent of the spores were inhibited by an aggregated sample of red cuprous oxide, whereas 93.6 per cent were inhibited by a non-aggregated sample. Likewise, in another test, with yellow cuprous oxide at 0.1 per cent copper, the aggregated sample inhibited 29.3 per cent of the spores, while the non-aggregated sample inhibited 93.1 per cent.

Effect of Cupric Oxide. The presence of black cupric oxide in red cuprous oxide darkens it and reduces its fungicidal value. For example, in one test a darkened sample containing 21.8 per cent cupric oxide inhibited 41.8 per cent of the spores, whereas a non-darkened sample inhibited 81.0 per cent. Horsfall, Marsh, and Martin (6) show that cupric oxide is much less fungicidal than cuprous oxide.

Seed-protective Value

As cuprous oxide is of much practical importance as a seed protectant, some of the samples of cuprous oxide of varying particle size and color were tested for their protective value on pea seeds.

Effect of Method of Manufacture. Four experiments were made, 3 in the greenhouse and climate laboratory and 1 in the field.

In the first experiment pea seed treated at 0.25 per cent dosage by weight were planted in the greenhouse and climate laboratory in soils with a high damping-off inoculum potential (Table 6). The data indicate that the best protective value against damping-off was exhibited by yellow cuprous oxide, the material of smallest particle size.

TABLE 6.—*Effect of particle size of cuprous oxides on their seed-protective value in soils of high inoculum potential in the greenhouse and climate laboratory*

Material[a]	Mean particle dia.	Average emergence	
		Greenhouse	Climate laboratory
	μ	*Per cent*	*Per cent*
None		0	11
Thermal oxide, T 1	2.93	26	49
Electrolytic oxide, R 3	1.95	25	
Electrolytic oxide, R 4	1.94	22	76
Electrolytic oxide, R 8	1.47	24	77
Yellow cuprous oxide, Y 1	0.94	28	82

[a] All materials used at a dosage of 0.25 per cent by weight.

In the second experiment the dosage of the various materials was reduced to 0.125 per cent to exaggerate differences in seed-protective value (Table 7).

TABLE 7.—*Effect of particle size of cuprous oxides on their seed-protective value in high inoculum-potential soils in the greenhouse and climate laboratory*

Material[a]	Mean particle dia.	Average emergence	
		Greenhouse	Climate laboratory
	μ	*Per cent*	*Per cent*
None	......	0.5	0.0
Thermal oxide, T 1	2.93	39.5	30.0
Electrolytic oxide, R 4	1.94	54.0	60.0
Electrolytic oxide, R 8	1.47	63.5	......
Yellow cuprous oxide, Y 1	0.94	62.5	73.0

[a] All materials used at a dosage of 0.125 per cent by weight.

These data show also the inverse correlation between particle size and seed-protective value of cuprous oxides; the material of largest particle size was lowest in value.

In the third experiment, thermal oxide T 1, electrolytic red oxide R 4, and yellow cuprous oxide Y 1 were tested at 0.0625, 0.125, 0.250, 0.500, and 1.000 per cent dosages by weight (Table 8). The data show that as the particle size decreases the dosage required for equal protection also decreases. The minimum load on the seed required to give maximum protection has been defined as the "minimum coverage dosage" (5). The amount of the 3 materials by weight to give the minimum coverage dosage was approximately 1.0 per cent for thermal cuprous oxide T 1, 0.5 per cent for electrolytic red cuprous oxide R 4, and 0.25 per cent for yellow cuprous oxide Y 1. On the basis of milligrams per square centimeter of seed surface exposed (5) these dosages become approximately 1.18 mg. for the thermal oxide, 0.593 mg. for the red oxide, and 0.297 mg. for the yellow oxide. The order of magnitude of seed protective value is, therefore, 1, 2, 4 for red-brown, red, and yellow cuprous oxides.

TABLE 8.—*Effect of particle size of cuprous oxides on their seed-protective value in soils of high inoculum potential in the greenhouse and climate laboratory*

Dosage by weight	Average emergence, per cent					
	Greenhouse[a]			Climate laboratory[b]		
	Thermal oxide T 1 (2.93 μ)	Electrolytic red oxide R 4 (1.94 μ)	Yellow cuprous oxide Y 1 (0.94 μ)	Thermal oxide T 1 (2.93 μ)	Electrolytic red oxide R 4 (1.94 μ)	Yellow cuprous oxide Y 1 (0.94 μ)
Per cent						
.0625	25	46	58	21	48	54
.125	37	56	65	44	65	78
.250	58	66	81	57	67	81
.500	60	81	87	65	73	80
1.000	74	83	86	71	76	70

[a] Average emergence of non-treated seeds was 5.0 per cent.
[b] Average emergence of non-treated seeds was 4.0 per cent.

To check the greenhouse and climate laboratory data, a field experiment was made, comparing red and yellow cuprous oxides at 0.25 per cent dosage by weight on peas. The average emergence in 5 replicate rod-row plots of 100 seeds each was 74.0 and 82.4 per cent, respectively, for the red and yellow cuprous oxides. The average emergence of the non-treated seeds was 50.0 per cent.

Effect of Fractionation. At first glance the above data appear at variance with those already published (1, 9) showing that thermal cuprous oxide is equal to red cuprous oxide in seed-protective value. The published data, however, were obtained with excess dosage. In the experiment cited above (Table 8), the 1.0 per cent dosage was an excess dosage and *at 1.0 per cent the thermal and red cuprous oxides were equal* in seed-protective value. The amount of material required to give the minimum coverage dosage for the red oxide was, however, only one-half that for the thermal oxide.

The screenings from the 1.0 per cent dosage of thermal oxide T 1 were weighed and found to account for approximately half of the quantity originally applied. This means that a dosage of only one-half per cent of the thermal oxide was actually on the seeds as planted. It means also that both thermal and red cuprous oxide showed essentially the same minimum coverage dosage of 0.593 mg. of cuprous oxide per square centimeter of pea seed surface. Apparently, the seeds had selectively fractionated the thermal oxide. The fraction that adhered was as effective as an equal load of red cuprous oxide. It seems probable that the mean particle diameter of the fraction that adhered was the same as that of the red cuprous oxide and that the large-size particles were in the fraction screened off.

These data confirm those on fungicidal value, showing that the thermal oxide contains a wide range of particle sizes and that the small ones obtained by fractionation are as effective as those in other cuprous oxides.

Effect of Aggregation. The relation of aggregation of particles to seed protective value of cuprous oxide was first observed in 1933. Up to that time commercial cuprous oxides mostly contained mineral oil as an antioxidant. The oil clumped the particles badly. Being oily, the clumps did not break down in the seed treater. Such lumpy samples were inferior to dusty samples in seed protective value. This was proved experimentally by extracting the oil from 4 commercial lots of red cuprous oxide in a Soxlet apparatus and comparing in the climate laboratory the seed-protective value on peas of the oily and oil-free lots. Eight tests were made with 25-seed lots in the climate laboratory at 15° C.

The average paired percentage emergence of peas treated with 4 oily and oil-free samples was as follows: 53.0 and 70.6, 81.6 and 84.0, 69.4 and 83.2, 69.1 and 82.3, respectively. These data show that if the effective particle size is increased with oil, protective value is reduced.

If the aggregation is attributable to other causes than the presence of oil, it seems less important in seed protection. The aggregation in the samples discussed under Fungicidal Value was not traceable to oil. These same samples were tested on peas and gave essentially equal protection as nonaggregated samples. Apparently, the aggregates were broken down by the impact of the seeds in the process of treating.

Effect of Cupric Oxide. The reduction in protective value of cuprous oxide when it oxidizes to cupric is so striking as to be evident in many cases to growers.

Studies were made in 1933 on the effect of black cupric oxide as a contaminant in red cuprous oxide on seed-protective value. Two approaches were used—(a) with samples contaminated by natural oxidation and (b) with samples artificially contaminated.

Several commercial samples that had oxidized in varying degrees to cupric oxide were obtained. The cupric oxide content was determined by the official method.[5] The improved method of Hurd and Clark (8) was not then available.

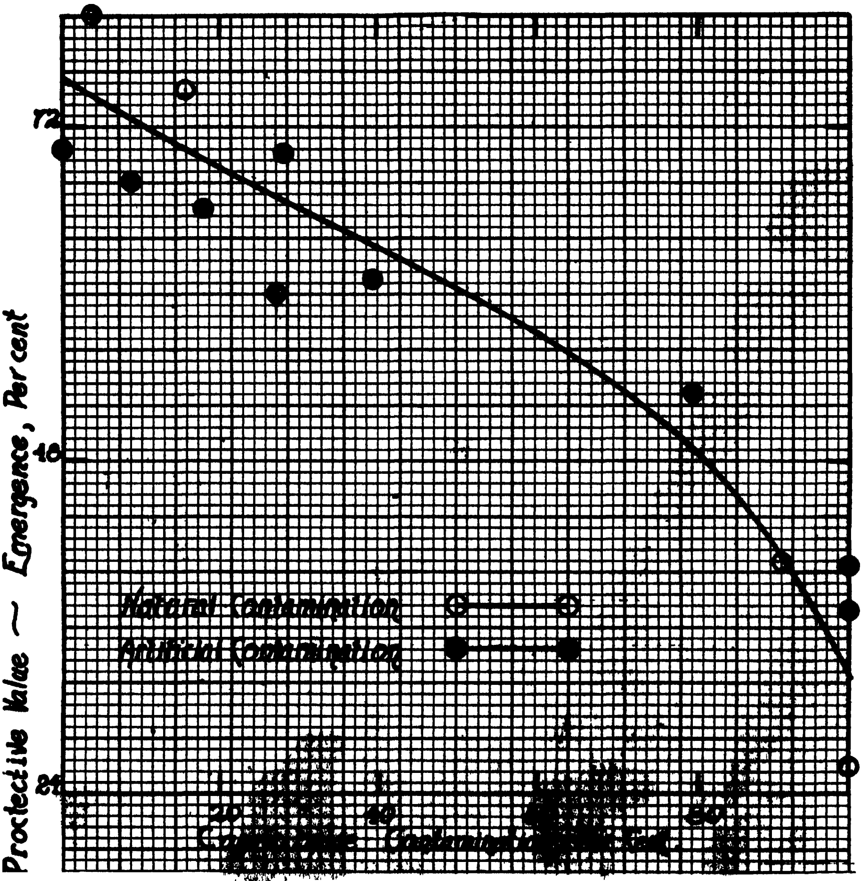

FIG. 1. Effect of contamination by cupric oxide on seed-protective value of cuprous oxide on peas. Curve drawn by inspection.

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[5] The writers are indebted to Messrs. Z. I. Kertesz and George Pearce of this station for the chemical determinations.

A portion of electrolytic oxide R 3 was burned in a muffle furnace to cupric oxide, which was then pulverized as well as possible in a mortar. The original red material was then contaminated in various proportions with the cupric oxide thus prepared. All of these materials were tested on peas in the climate laboratory at 15° C. in 4 tests totalling 16 25-seed replicates for each material.

It is interesting that either approach gives points which fall approximately on the same curve (Fig. 1), which shows that seed-protective value falls off steadily as the cupric oxide content increases.

Foliage-protective Value

Black Spot of Roses. The data on fungicidal and seed-protective value indicate that yellow cuprous oxide is about twice as potent as red cuprous oxide. These materials were compared on roses in 1938 for the control of black spot caused by *Diplocarpon rosae* Wolf. They were used at a concentration to give ½ and 1½ ounces of metallic copper in 50 gallons of water. The test was begun on May 4 and sprays were applied weekly until July 29. Data were taken at intervals on the number of plants infected with black spot (Table 9). They show that the yellow cuprous oxide gave the same control of black spot as the red cuprous oxide with one-third the dosage of copper, thus confirming the data on fungicidal and seed-protective value.

TABLE 9.—*Protective value of red and yellow cuprous oxides against rose black spot, 1938*

Material[a]	No. of plants examined	Plants showing black spot on		
		June 30	July 15	Aug. 2
		Per cent	*Per cent*	*Per cent*
Red cuprous oxide 1½ oz.–50 gals. + ⅛ per cent soluble cottonseed oil	710	6.3	10.4	31.0
Yellow cuprous oxide ½ oz – 50 gals. + ⅛ per cent soluble cottonseed oil	650	8.1	15.7	27.0

[a] Fourteen sprays, May 4–Aug. 2.

Early Blight of Tomatoes. Tomatoes were sprayed in 1938 with red-brown, red, and yellow cuprous oxides and with Bordeaux for control of *Alternaria solani* (Ell. and Mart.), using the technique described elsewhere (7). All sprays were used at a concentration of 1 lb. of copper to 50 gal. of water, and were applied at a rate to give 6 pounds of copper per acre. Two types of weekly sprayings were made, *i.e.*, early in the season, up to July 29, and late in the season, commencing July 29.

For the early-season test, data were taken on September 2, 5 weeks after the last spray application. These data included the number of leaves killed per plant and the number of stem-end-rot lesions (Table 10).

It is clear from the data that yellow cuprous oxide gave better protection

TABLE 10.—*Protective value of red-brown, red, and yellow cuprous oxides against Alternaria solani on tomatoes, 1938*

Material[a]	Mean particle dia.	Dead leaves per plant[b]	Fruits infected[b]
	μ	*No.*	*Per cent*
Non-sprayed	......	129	9.9
Red-brown cuprous oxide	2.93	84	7.7
Red cuprous oxide	1.94	64	6.6
Yellow cuprous oxide	0.94	59	1.1
Bordeaux	... .	59	0.6

[a] All materials were used at a concentration of 1 lb. copper to 50 gal. of water. Eight sprays were applied, June 10–July 29.

[b] Reading made on Sept. 2.

against stem and leaf infection than red cuprous oxide and that red cuprous oxide gave better protection than red-brown cuprous oxide.

Data from the late-season test (Table 11) were taken variously; percentage dead leaves per plant, number of stem-end-rot infected fruits, average green weight per plant, and index of disease based on an individual reading on each of the 80 plants in the four replicate plots. From these data it is again clear that yellow cuprous oxide gave much better disease control than red cuprous oxide and closely approached Bordeaux mixture in disease control.

TABLE 11.—*Protective value of red and yellow cuprous oxide against Alternaria solani on tomatoes, 1938*

Material[a]	Mean particle dia.	Green wt. per plant	Blight index[b]	Dead leaves per plant	Fruits infected
	μ	*Lb.*		*Per cent*	*Per cent*
Non-sprayed	... .	1.18	4.98	66.4	53.4
Red cuprous oxide	1.94	1.89	3.58	47.0	31.0
Yellow cuprous oxide	0.94	2.39	2.65	33.8	15.3
Bordeaux		2.43	2.51	30.1	15.9

[a] All materials were used at a concentration of 1 lb. of copper to 50 gal. of water. Five sprays were applied, July 29–Sept. 9.

[b] Based on scoring 80 plants for each treatment. Maximum index = 5.00.

The order of magnitude for the foliage-protective-value tests on tomatoes cannot be calculated because dosage tests were not made.

Aggregation of Particles in Foliage Dusts. The data presented on the relation of effective particle size to seed-protective value of cuprous oxide show that as the effective particle size is increased by aggregation, as by oil, for example, the seed-protective value decreases. Aggregation should bear the same relationship to the foliage-protective value of dusts. An exploratory study was made of this relationship.

Samples of commercial red copper oxide and copper oxychloride dusts were first examined. Visually, the uniform color of the dusts indicated a uniform distribution of the copper particles. When small amounts of the dusts, however, were smeared across white paper with a spatula, streaks of red and blue were visible in the red copper oxide and copper oxychloride dusts, respectively. This streaking showed that all the aggregates of the copper particles had not been broken up and dispersed during the process of mixing the dusts. Copper-lime dusts also showed streaking when smeared on filter paper subsequently moistened from below.

Dusts were next prepared in a ball mill in 2 ways: (1) all the ingredients were milled together; and (2) the red copper oxide and copper oxychloride were milled alone, first, and then the rest of the ingredients added, and the whole milled again. The spatula streak test showed less streaking in the dusts where the copper materials were milled alone, first, than in the dusts where all the ingredients were milled together. This showed that milling the copper materials alone broke up the aggregates of particles and thus resulted in a more nearly uniform dispersion when the other materials were added.

Two dusts were next prepared in identical fashion, using two samples of red copper oxide—one containing aggregates and the other free of aggregates. The spatula test showed numerous streaks in the dust prepared from the aggregated sample and almost no streaks in the dust prepared from the sample free of aggregates. Thus, in preparing dusts, it is essential that the copper ingredients be free of aggregates and that the copper particles be uniformly dispersed to obtain full expression of the foliage-protective value.

The aggregates of particles of toxicant in the present copper dusts may explain the poorer disease control exhibited by these dusts compared to equivalent sprays. This point is being investigated in detail.

DISCUSSION

The data presented on fungicidal and protective value show that as the particle size of cuprous oxide is decreased these values are increased. Moreover, these values increase as the color shifts from red-brown through red and orange to yellow and as the wave length of reflected light decreases from 6440 to 5950 Å. As the color of cuprous oxides is a phenomenon associated with particle size (17), color can be used as a reliable index of efficiency.

The data on seed protective value are at variance with those reported (1, 9) showing that thermal cuprous oxide was as good a seed protectant as electrolytic red cuprous oxide. These variations may be explained on the basis of the technique used for evaluating the materials. In the recent reports (1, 9) the materials were used in excess and what did not adhere was screened off, thus giving the materials every chance. In the work reported in this paper the materials were used at regulated dosages by weight of seed and a range of dosages were employed. The data in table 8 are very illustrative of the effect of varying the dosage on seed-protective value.

It has been reported recently (1) that cuprous oxide material should be fine enough to pass a 325-mesh screen and that additional fineness did not improve its fungicidal value. A 325-mesh screen does not measure fineness of particles; it measures only coarseness. It means only that materials having a mean particle diameter of over 44 μ will not pass through it. Twentyman (16) has pointed out that mesh does not actually give a measure of the fineness of the particles that pass through it and, further, gives no indication of the probable efficiency. The data presented in tables 3 and 4 show that the fungicidal value of cuprous oxides is determined by their particle size or fineness. These data are in line with those reported by Twentyman (16) on bunt of wheat. Working with 6 fractions, obtained by air flotation, of a standard copper carbonate dust he showed that the coarsest fraction (50 μ) allowed 4½ times as much smut as the finest fraction (2.5 μ).

In this paper the relationship of particle size to fungicidal, seed-protective, and foliage-protective value has been established. Particle size in itself, however, is not the whole story. Wilcoxon and McCallan (18) have pointed out in their work on particle size of sulphur that a reduction in particle size results in an increase in the number of particles per unit weight and that fungicidal value is correlated with the number of particles.

An increase in the number of particles per unit weight results in an increase in the area of chemically reactive surface. The relation of particle size to number of particles and area of reactive surface per unit weight is shown in table 12. It is readily seen from the data that fungicidal value may be correlated with number of particles and area of reactive surface, for they are determined by particle size—the larger the particle size the less the number of particles and the smaller the area of chemically reactive surface, and *vice versa.*

TABLE 12.—*Relation of particle size to number of particles and chemically reactive surface area per unit weight*

Material	Mean particle diameter	No. of particles per gram	Reactive surface area per gram[a]
	μ	$\times 10^7$	$cm.^2$
Thermal cuprous oxide, T 1	2.93	125	337
Electrolytic red cuprous oxide, R 4	1.94	439	417
Yellow cuprous oxide, Y 1	0.94	4,034	1,120

[a] Assuming that the particles are spheres.

The probable explanation for the increased fungicidal and protective value of the small particles is that the reactive surface area is increased. This accelerates the rate of solubility, or rather the rate of availability (6) of the copper to the germinating spore. In conversation some physical chemists maintain that the final equilibrium of soluble copper is higher with the small particle than with the large particle samples. Others maintain that this does not work out practically. The writers did not test this point.

It would appear that yellow cuprous oxide is worthy of trial as a new fungicide because it appears to be close to the fungicidal and foliage-protective value of Bordeaux. Yellow cuprous oxide is, of course, the active ingredient in the Raleigh (11) mixture, which is prepared by reducing copper sulphate to cuprous oxide with glucose and lye. This mixture has given promising field results; yellow cuprous oxide powder also should give promising results.

Red cuprous oxide was difficult to stabilize commercially against oxidation. Because of its smaller, more active particles, yellow cuprous oxide has been even more difficult to stabilize and at present is stabilized at 50 per cent copper content. This yellow material is conditioned for spraying. As the pure material is available only in experimental lots commercial seed treatment must still depend upon the red material.

The color that yellow cuprous oxide imparts to foliage may be misleading. Being yellow, the spray film filters some of the blue rays from the chlorophyll, making it seem lighter green than normal. This is particularly apparent when a comparison is made with a blue spray residue that filters the yellow rays from the chlorophyll, making it seem a deeper green than normal.

The color change in cuprous oxide from red through orange to yellow, as the particle size decreases, is associated with a shortening of the wave length of reflected light. According to Ridgway (12), a representative red emits light at 6440 Å, an orange at 5980 Å, and a yellow at 5770 Å. It appears then that, as the wave length of reflected light becomes shorter, cuprous oxide becomes a more potent fungicide.

The wave length of the red element in the red-brown thermal cuprous oxide is about the same as the wave length of the red in the electrolytic red oxides.

With further regard to the average particle size of red and yellow oxides, it is interesting that the particles of yellow mercuric oxide are smaller than those of red mercuric oxide. Moreover, if aggregates of red oxide of mercury, red oxide of lead, and red oxide of iron in water suspension are broken down with a detergent the color changes markedly toward yellow. These tests indicate that decrease in wave length of reflected light with decrease in particle size is a general phenomenon among red oxides of metals.

Apropos of the question of correlation between laboratory work on fungicidal value and field work on protective value, it is worthy of note that all of the samples of cuprous oxide occupied the same relation to each other in the field on rose foliage against *Diplocarpon rosae* and on tomato foliage against *Alternaria solani,* in the greenhouse and field on pea seeds against *Pythium ultimum,* as they did in the laboratory against *Macrosporium sarcinaeforme.*

SUMMARY

This paper presents the results of experiments made on the relation of particle size and color of cuprous oxides to fungicidal value, seed-protective value, and foliage-protective value.

A technique is described for measuring rapidly the particle size of cuprous oxide suspensions.

The fungicidal value (ability to inhibit spore germination) was measured by a modification of the well-known slide-testing technique, using a precision sprayer. The protective value (ability to prevent infection) was measured on seeds and foliage.

The color of cuprous oxides is a function of particle size. The normal range in color and particle size extends from a red at about 2.57 μ mean particle diameter through orange to yellow at about 0.94 μ mean particle diameter. The presence of black cupric oxide imparts a dark shade. The mean particle diameter of thermal cuprous oxide ranges up to 2.93 μ.

Mean particle diameter is a more exact measure of the fineness of powders than is mesh.

The fungicidal and protective values vary inversely as the particle size. They increase as the color shifts from red through orange to yellow. The presence of cupric oxide appears to reduce the fungicidal and protective values probably because it increases the mean particle diameter and because it dilutes the cuprous oxide.

As the color of cuprous oxides is a function of particle size, the fungicidal and protective values can be forecast with reasonable accuracy from the color. That is, a series of cuprous oxides can be arranged in their approximate order of performance from their color.

The probable explanation for the increased fungicidal and protective values of cuprous oxides of small particle size is that the area of chemically reactive surface per unit of weight is increased and that the rate at which soluble copper is presented to the germinating spore is increased.

Apropos of the significance of laboratory tests in fungicide research, it is noteworthy that the various samples of cuprous oxide occupied the same relation to each other in the field on rose and tomato foliage against *Diplocarpon rosae* and *Alternaria solani,* in the greenhouse and field on pea seeds against *Pythium ultimum,* as they did in the laboratory against *Macrosporium sarcinaeforme.*

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INHIBITION OF VIRUS ACTIVITY BY INSECT JUICES

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INTRODUCTION

In the case of insect vectors in which viruses exhibit an incubation period and in which they are retained for a considerable time, investigations have been made to ascertain if the viruses multiply in the insects. Merrill and TenBroeck (21) have demonstrated that the virus of western equine encephalomyelitis multiplies in the mosquito, *Aedes aegypti* L. Fukushi (14), working with dwarf disease of rice, and Kunkel (17), with aster yellows, interpret their experiments as indicating that the viruses of these diseases multiply in their vectors. Freitag (12) and Bennett and Wallace (3), however, conclude from experiments on curly-top virus that multiplication in the beet leaf hopper is improbable. The potato yellow-dwarf virus has an incubation period in the clover leaf hopper, *Aceratagallia sanguinolenta* Prov. (5), and it is also retained a long time by this vector (6). It was thought that the possible multiplication of this virus in the clover leaf hopper could be investigated by means of the primary lesions that the virus produces in the leaves of *Nicotiana rustica* L. (7). Attempts to obtain primary lesions in *N. rustica* by inoculation with the juice of viruliferous leaf hoppers were, however, entirely negative.

With one exception (20), other investigators (27, 25, 13) have failed in similar attempts to infect plants with juice from insects carrying other viruses. In some cases the failure to obtain infection may be attributed to the fact that the viruses are not readily transmissible from plant to plant by mechanical methods. There has been, however, no satisfactory explanation for the case of other viruses that are transmitted easily from plant to plant but not from insect to plant. Hamilton (15) observed that the addition of juice from crushed aphids, *Myzus persicae* Sulz., to Hy. III virus rendered the mixture noninfectious. She suggested that the insect juice had a deleterious effect on the viability of the virus.

The experiments presented in this paper show that clover leaf-hopper juice inhibited the infectivity of juice from yellow-dwarf *Nicotiana rustica* plants. The inhibitor (or inhibitors) in the insect juice also suppressed the infectivity of several other viruses, including that of tobacco mosaic. Since yellow-dwarf virus is rather unstable, tobacco-mosaic virus was employed in an investigation of the action and nature of the inhibitor. It was hoped that this study would make it possible to eliminate the inhibitor from the juices of viruliferous leaf hoppers and thus facilitate research on the virus in the insect. Although methods of separating tobacco-mosaic virus from inhibitor were found, the instability of the yellow-dwarf virus has thus far prevented their use with this virus.

MATERIALS AND METHODS

Clover leaf hoppers, free from extraneous material, were counted, weighed, and inactivated by placing them at – 14° C. for 30 minutes. They were then ground in a mortar and the pulp immediately suspended in 0.1 M phosphate buffer at pH 7.0. The suspension was centrifuged to remove the coarse material and the creamy-white supernatant used at once in various experiments. The concentration of the inhibitor in such solutions has been expressed in terms of leaf-hopper weight per cc. For example, the description of a solution as having an inhibitor concentration of 18 mg. per cc means that 1 cc. of buffer solution contained the juice obtained from 18 mg. of clover leaf hoppers. The average weight of a single leaf hopper varied in different samples from 1.5 to 1 8 mg. Unless otherwise specified, 0.1 M phosphate buffer at pH 7.0 was used in making all suspensions and dilutions. All hydrogen-ion determinations were made by means of a glass electrode.

The infectious juice used in the experiments was prepared from leaves of Turkish tobacco, *Nicotiana tabacum* L., diseased with tobacco mosaic. The leaves were frozen and ground, and the juice expressed through cheesecloth. The coarser material was removed by centrifugation and the juice stored frozen in small corked test tubes. This juice, or certain dilutions of it in buffer, was mixed with the insect preparation. The effect of different treatments on the inhibitor was determined by the infectivity of the various mixtures tested on from 28 to 36 half-leaves of Early Golden Cluster beans, *Phaseolus vulgaris* L. The samples in each experiment were paired in all possible ways and applied to the half-leaves according to the method of randomization described by Youden (33) as incomplete blocks. In this case, however, the two halves of the same leaf constituted a block. The excess inoculum was rinsed from the leaves with tap water. The lesions were counted 3 to 6 days after inoculation.

EXPERIMENTS

The Nonspecific Nature of the Reaction

As stated in the introduction, the juice from leaf hoppers carrying the potato yellow-dwarf virus was noninfectious to *Nicotiana rustica* leaves. Juice from about 2000 insects was applied to leaves in various ways in the tests. In one experiment each live leaf hopper was placed on a leaf dusted with carborundum, crushed beneath a spatula, and the juices immediately rubbed into the leaf. In control inoculations juice from diseased *N. rustica* leaves produced numerous primary lesions.

In order to determine whether leaf-hopper juice would inhibit the virus, 10 macerated insects were added to each cc. of a 10^{-2} dilution of infectious plant juice. The mixture failed to produce a single lesion on 20 half-leaves of *Nicotiana rustica,* while the corresponding insect-free preparation was highly infectious (Table 1).

TABLE 1.—*Inhibiting action of clover leaf-hopper juice on plant viruses*

Virus	Concentration of juice from diseased plants	Test plant	Total lesions in 20 half-leaves or 20 leaves inoculated by virus solution containing:	
			10 leaf hoppers per cc.	No leaf hoppers
Potato yellow dwarf	10^{-2}	*Nicotiana rustica*	0	1156
		Nicotiana rustica	0	180
Tobacco mosaic	10^{-3}	*Nicotiana glutinosa*	5	561
		Bean	0	619
Potato X	10^{1}	Turkish tobacco	8	845
Turnip mosaic	10^{0}	Turkish tobacco	4	786
Tobacco necrosis	10^{-1}	Cowpea	301	3707
Tobacco ring spot #1	10^{0}	Cowpea	12	1366

It then became of interest to ascertain whether the juice of clover leaf hoppers would inhibit other plant viruses. Suitable dilutions of juices from plants infected with the viruses of tobacco mosaic, potato X, turnip mosaic, tobacco necrosis, or tobacco ring spot No. 1, with and without the addition of 10 macerated leaf hoppers per cc., were compared on opposite half-leaves or opposite leaves of appropriate test plants. Turkish tobacco, *Nicotiana tabacum*; cowpea, *Vigna sinensis* Endl. var. Black; bean, *Phaseolus vulgaris* var. Early Golden Cluster; *N. rustica*; and *N. glutinosa* L. were used in the tests. The results, presented in table 1, indicate that clover leaf-hopper juice has a general inhibitory action. The data demonstrated that the inhibitor is not specific and suggested the possibility of investigating its nature and action by studying its effect on inhibition of tobacco-mosaic virus on bean. Accordingly, tobacco-mosaic virus was employed in all subsequent experiments.

Experiments were conducted to determine whether the juice of other insect vectors of viruses likewise contain an inhibitor. The insects used were mosquitoes, *Aedes aegypti;* aphids, *Aphis rumicis* L., *Macrosiphum pisi* Kaltenbach, *M. solanifolii* Ashm., and *Myzus persicae;* and leaf hoppers, *Eutettix tenellus* Baker and *Macrosteles divisus* Uhler. A 10^{-3} dilution of the infectious juice from a mosaic-diseased tobacco plant was added to the macerated pulp of the insect under investigation so that the concentration of the insect juice was 15.5 mg. per cc.—the approximate weight of 10 clover leaf hoppers. This suspension was applied to 20 half-leaves of bean, and the virus preparation to which no insect juice had been added was applied to the opposite half-leaves. Although there was some seasonal variation in the susceptibility of the test plants, the data presented in table 2 indicate that the juices of aphids, leaf hoppers, and mosquitoes have the same inhibiting action on the virus. The results suggest that the inhibitory effect may be a general property of insect juices.

TABLE 2.—*Inhibition of tobacco-mosaic virus by juice of various insect vectors of viruses*

Insect	Total lesions in 20 half-leaves of bean inoculated with tobacco-mosaic-virus solution containing:	
	15.5 mg. of insect per cc.	No insects
Aceratagallia sanguinolenta	0	619
Aedes aegypti	0	250
Aphis rumicis	1	647
Eutettix tenellus	0	1885
Macrosiphum pisi	0	461
Macrospihum solanifolii	0	545
Macrosteles divisus	0	318
Myzus persicae	0	641

Effect of Dilution on the Inhibitor and the Virus

Dilution studies upon the inhibitor and the virus were undertaken in order to measure the activity of the inhibitor and to elucidate its mode of action. After preliminary tests, a stock solution of the inhibitor was prepared with a concentration of 17.94 mg. per cc. Mixtures were then prepared each of which contained a 1 : 250 dilution of infectious juice and one or another of various dilutions of inhibitor solution. Each mixture and a 1 : 250 dilution of infectious juice to which no insect juice had been added was applied to the leaves of 15 bean plants. This and the following experiments are the only ones in which solutions were not tested on half-leaves according to the incomplete block arrangement. The total number of lesions obtained with the solution having no inhibitor was 12,410, and the reduction in the number of lesions obtained with each concentration of inhibitor was calculated as a percentage of 12,410 and plotted in figure 1. It is apparent from the curve obtained that about 0.15 mg. of leaf hopper per cc. reduces the lesions by 50 per cent, and, since the inhibitor doubtless constitutes only a fraction of the leaf-hopper weight, it must be a very active substance. The experiment was repeated twice with similar results.

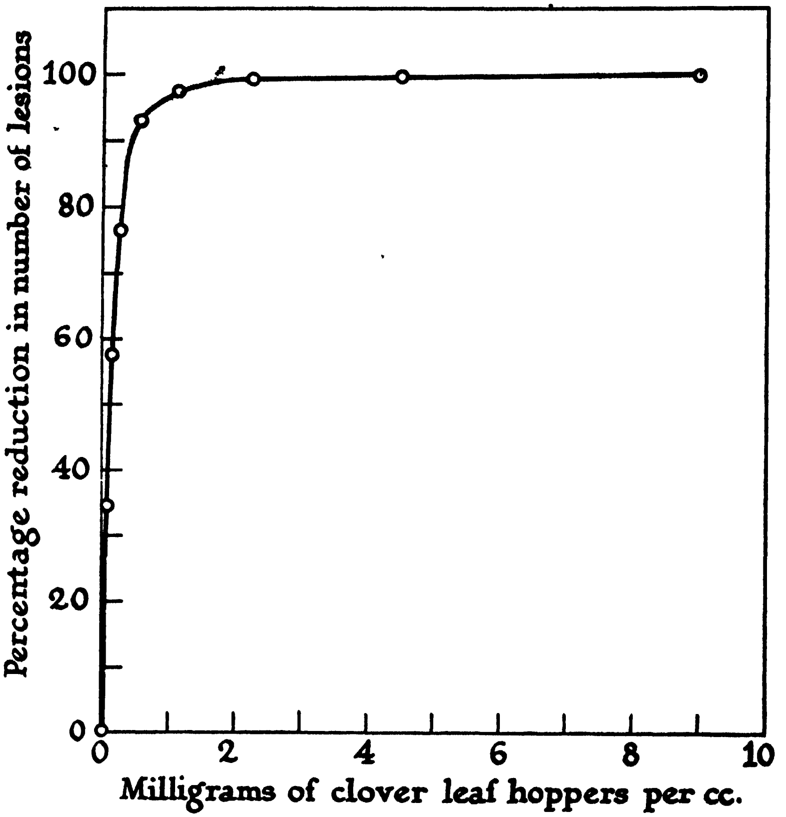

FIG. 1. Dilution curve of the inhibitor. The reduced numbers of lesions produced in 30 bean leaves by a 1: 250 dilution of infectious juice in the presence of various concentrations of inhibitor are expressed as percentages of the number (12,410) produced by a 1: 250 dilution of the juice without inhibitor.

The full effect of any concentration of inhibitor is exerted immediately after it is mixed with the virus solution. Leaves inoculated with the most concentrated inhibitor solution used showed some injury. These facts suggested that the effect of the inhibitor is on the host plant rather than on the virus. Accordingly, an experiment was designed to determine whether the percentage reduction in lesions depended only upon inhibitor concentration. If the percentage reduction in lesions were independent of virus concentration it would seem certain that the inhibitor acted chiefly upon the plant and little or not at all upon the virus. A 10^{-1} dilution of the infectious juice was compared by the half-leaf method with the same solution in which the inhibitor concentration was 0.25 mg. per cc.; 10^{-2}, 10^{-3}, and 10^{-4} dilutions, with and without insect juice, were compared in the same way. The data from 3 replications of this experiment are presented in table 3. The general trend of the results is for the percentage reduction to increase slightly as the virus concentration decreases. The virus concentration in the first mixture is 1000 times that of the 4th, yet the percentage reduction varies comparatively little. Although this slight increase in the percentage reduction indicates some action of the inhibitor upon the virus, it seems probable that the principal effect is upon the plant.

TABLE 3.—*Action of a single concentration of inhibitor (0.25 mg. per cc.) on various dilutions of tobacco-mosaic virus*

Dilution of infectious juice	Experiment 1			Experiment 2			Experiment 3		
	Total lesions in 28 half-leaves of bean		Reduction in lesions	Total lesions in 36 half-leaves of bean		Reduction in lesions	Total lesions in 36 half-leaves of bean		Reduction in lesions
	With inhibitor	Without inhibitor		With inhibitor	Without inhibitor		With inhibitor	Without inhibitor	
			Per cent			*Per cent*			*Per cent*
10^{-1}	2996	6633	54.8	1045	4273	75.5	1226	4645	73.6
10^{-2}	1350	2782	51.5	610	2587	76.4	1160	3050	62.0
10^{-3}	494	789	37.4	177	1075	83.5	366	1146	68.0
10^{-4}	81	272	70.2	41	404	89.9	47	232	79.8

If the principal action of the inhibitor is on the leaf, then dilution of certain virus-inhibitor mixtures should increase their infectivity. A mixture, with a pH of 6.9, containing undiluted infectious juice and an inhibitor concentration of 15.25 mg. per cc. was prepared. Seven dilutions of this mixture in buffer and one dilution of the infectious juice to which no insects had been added were compared on half-leaves of bean. Dilution tests also were conducted with mixtures prepared in the same way but having virus concentrations only 1/10 and 1/100 of that in the first mixture. All 3 mixtures were tested on beans from the same lot on the same day, so that the 3 dilution curves obtained (Fig. 2) may be compared one with the other. In each case the infectivity of the mixture increased until the concentration of the inhibitor was only about $10^{-2.5}$ its original concentration, or about 0.05 mg. per cc. At higher dilutions the curve begins to follow the normal dilution curve for the tobacco-mosaic virus. It is interesting that the mixture containing the lowest concentration of virus gave no infections until it had been diluted. The experiment was repeated with similar results, and the same phenomenon was demonstrated, incidentally, in several subsequent experiments. In the light of these results, concentrated suspensions of

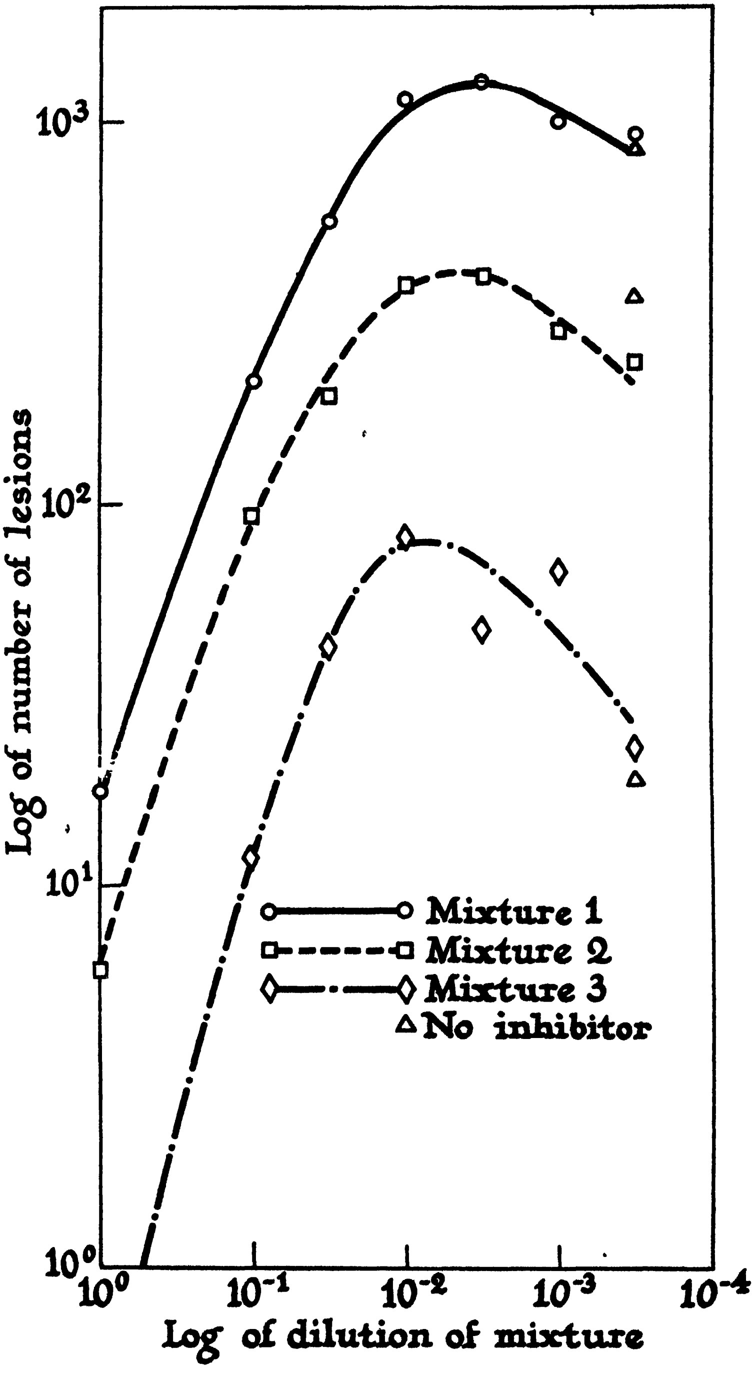

FIG. 2. Increase in the infectivity of virus-inhibitor mixtures upon dilution. All mixtures before dilution contained the same concentration of inhibitor (17.94 mg. per cc.). Mixture 1 contained 100 times as much virus as mixture 3, and mixture 2 ten times as much.

macerated viruliferous insects would seem less likely to be infective than would more dilute solutions.

The results of the previous experiments indicate that the inhibitor does not destroy the virus during the interval between the preparation of the mixtures and their testing on beans. In order to determine if the inhibitor would destroy the virus during longer intervals of contact, 7 samples of an inhibitor solution with a concentration of 17 mg. per cc. were mixed with equal volumes of infectious juice. After being held for periods up to 24 hours, the mixtures were diluted 1:50 in buffer, and inoculated on bean leaves. The mixtures were prepared at different times but tested for infectivity at the same time. The total number of lesions produced by each sample on 35 half-leaves is plotted in figure 3. The data of a second experiment with 8 samples were adjusted so that the total number of lesions produced by 7 samples was the same as that of the corresponding samples in the first experiment. The corrected values also are plotted in figure 3, and a straight line fitted to all 15 points. The slope of this line (4449×10^{-7}) is not significantly different from zero and indicates there was no appreciable destruction of the virus during 24 hours.

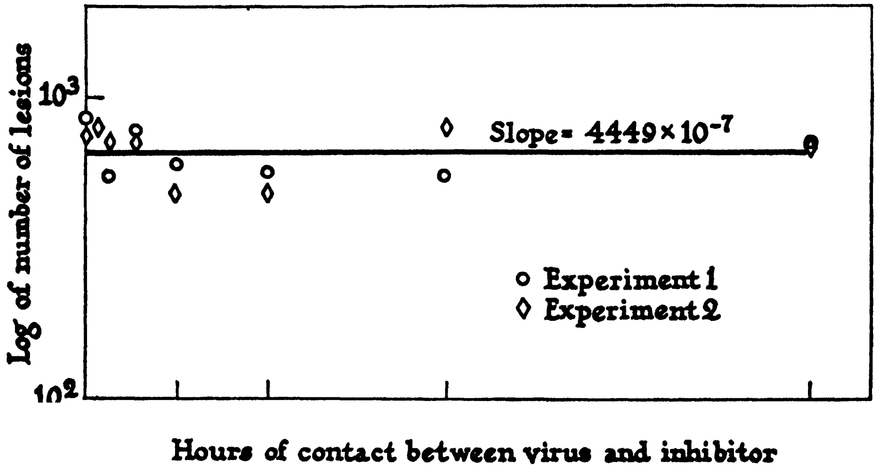

FIG. 3. Stability of tobacco-mosaic virus in the presence of the inhibitor.

Detection of Virus in Virus-inhibitor Mixtures

The dilution studies demonstrated that virus could be detected in certain virus-inhibitor mixtures simply by dilution. Other investigations were undertaken for the purpose of finding better methods of detecting virus in noninfectious or slightly infectious virus-inhibitor mixtures. These studies also have contributed information upon the nature of the inhibitor.

Dialysis of the Inhibitor. It seemed possible that the inhibitor could be removed from virus-inhibitor mixtures by dialysis. An inhibitor solution with a concentration of 3.3 mg. per cc. was dialyzed against buffer in a

Kunitz-Simms (16) apparatus for 24 hours at room temperature. A control nondialyzed sample of the same solution was stored for 24 hours at room temperature. A third sample consisted of an inhibitor solution of the same concentration freshly prepared when the first sample was removed from the dialyzing apparatus. These solutions were then mixed 1: 1 with a 1: 125 dilution of infectious juice and the infectivities of the mixtures compared with each other and with a 1: 250 dilution of infectious juice to which no inhibitor had been added. If the inhibitor was unaltered by dialysis or storage, the amount used was expected to reduce the infectivity of the virus solution to about 5 per cent of what it otherwise would have been. The total number of lesions obtained on the 36 half-leaves inoculated with each sample is presented in table 4. The data indicate that the inhibitor is not readily dialyzable through a cellophane membrane under the conditions of the experiment and show that the inhibitor is stable over a period of 24 hours. The results of a second experiment, also presented in table 4, were confirmative.

TABLE 4.—*Effect of dialysis upon the inhibitor*

Experiment	Number of half-leaves inoculated	Lesions produced by a mixture containing infectious juice at 1: 250 and			
		An inhibitor concentration of 1.65 mg. per cc.			No inhibitor
		Inhibitor freshly extracted	Inhibitor stored at 25° C. for 24 hours	Inhibitor dialyzed 24 hours	
1	36	99	94	231	2277
2	28	127	80	76	1418

Thermolability of the Inhibitor. An experiment was designed to compare different dilutions of the inhibitor with inhibitor solutions subjected to various temperature treatments. The experiment permitted a quantitative estimation of the effect of the heat treatment on the inhibitor. The temperature treatments were carried out on inhibitor solutions with a concentration of 3.6 mg. per cc. Each solution was added to an equal volume of a 1: 125 solution of infectious juice. The 12 solutions in the experiment, therefore, contained a 1: 250 dilution of infectious juice and various amounts of inhibitor depending upon the dilution or the heat treatment. The heat treatments were carried out by immersing 70 × 5 mm. corked test tubes, containing 1 cc. of the solution, in a water bath held within 0.1° C. of the desired temperature. At the end of the 10-minute treatment, the tubes were immersed in cold water. The total number of lesions produced by each solution on 33 half-leaves is presented in table 5. By plotting the data for the first 6 solutions—those containing known amounts of inhibitor—in the form of a dilution curve, the data for the other 6 solutions may be interpreted roughly in terms of the amount of inhibitor destroyed by each treatment. The values obtained in this manner are plotted in figure 4.

TABLE 5.—*Effect of heat treatment upon the inhibitor*

Solution	Concentration of inhibitor (mg. per cc.)	Temperature treatment of inhibitor for 10 minutes (°C.)	Total lesions in 33 half-leaves	
			Exp. 1	Exp. 2
1	1.8	No treatment	55	96
2	0.9	"	338	245
3	0.45	"	557	511
4	0.225	"	1128	1392
5	0.1125	"	1440	1460
6	none	"	1256	2110
7	1.8	100	855	1883
8	1.8	90	917	1395
9	1.8	80	957	1307
10	1.8	70	466	449
11	1.8	60	121	136
12	1.8	50	65	84

The results of a second experiment, presented in table 5 and figure 4, are in general agreement with those of experiment 1. The inhibitor is apparently readily destroyed by heat.

In order to determine if the infectivity of virus-inhibitor mixtures could be increased by heating, samples having an inhibitor concentration of 5.08 mg. per cc. and a virus concentration 1/3 that of infectious juice were treated at 8 different temperatures between 90° and 55° C. for 10 minutes.

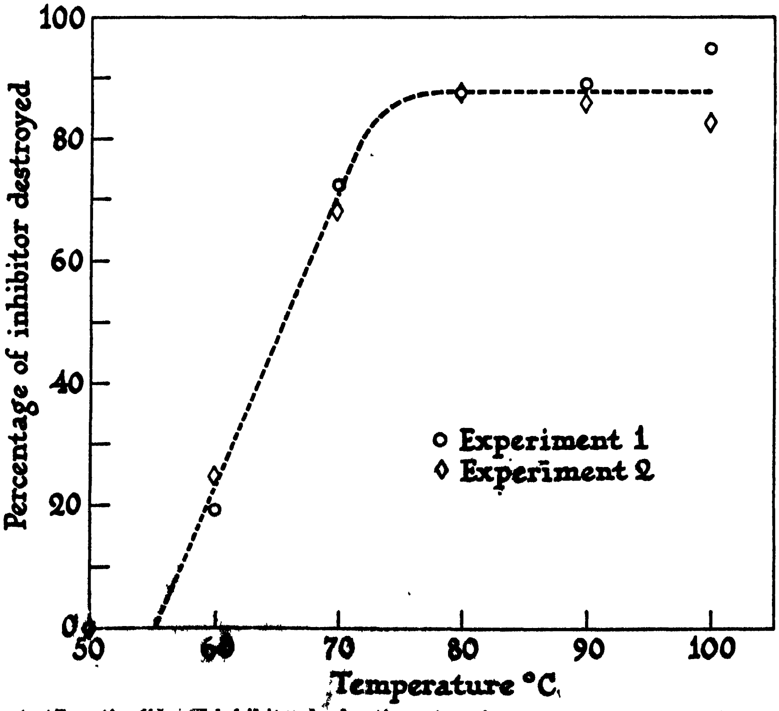

FIG. 4. Inactivation of inhibitor by heating at various temperatures for 10 minutes.

After being heated, these samples were diluted 1/3 in buffer to bring the inhibitor content to 1.69 mg. per cc., at which concentration the inhibitor should, if not inactivated by the heat treatment, almost completely suppress lesions. When the samples were compared on bean leaves, it was found that the infectivity increased with the temperature until 70° C. was reached. The infectivity of solutions treated at 75° C. or higher was markedly lower.

Effect of H-ion Concentration on the Inhibitor. In an experiment designed to determine the effect of hydrogen-ion concentration on the inhibitor, 11 concentrated solutions of the inhibitor in distilled water were adjusted to the desired H-ion concentration by the addition of equal volumes of complex buffer mixtures described by Best and Samuel (4). The hydrogen-ion concentrations of the inhibitor solutions were determined immediately and after a period of 24 hours' storage at room temperature. The solutions were then adjusted to approximately pH 7.0 by adding an equal volume of 0.1 M phosphate buffer. In the case of the most alkaline solution and the 2 most acid solutions, a preliminary adjustment with 0.2 N HCl or NaOH was made to bring the solution almost to the neutral point. To each of the neutral solutions was added an equal volume of a 10^{-2} dilution of infectious juice in buffer. These dilutions were calculated to bring the concentration of the inhibitor, if unaltered by the treatment, to 1.8 mg. per cc., an amount that would almost completely prevent infections. The 11 solutions and a control solution to which no inhibitor had been added were compared on bean leaves. The experiment was repeated twice; once with *Agallia constricta* Van Duzee as a source of inhibitor. The total number of lesions produced by each solution on 33 half-leaves is presented in table 6.

TABLE 6.—*The effect of hydrogen-ion concentration on the inhibitor*

Inhibitor solution	pH[a]		Lesions produced in 33 half-leaves after virus was added to solutions readjusted to pH 7.0		
	At beginning of storage	At end of 24 hours' storage	Experiment 1	Experiment 2	Experiment 3
1	1.32	1.31	1580	1320	825
2	2.35	2.38	2055	1748	1774
3	3.29	3.43	2655	1377	2663
4	4.21	4.55	1020	672	352
5	5.18	5.46	934	687	217
6	6.13	6.05	799	406	189
7	6.97	6.88	249	260	155
8	8.34	7.57	441	435	235
9	9.12	8.72	718	365	700
10	9.54	9.33	1020	499	1546
11	11.61	11.87	3253	2385	2904
12	No inhibitor and no pH treatment		3068	4188	3294

[a] The pH data are those for experiment 1. The pH data for experiments 2 and 3 were practically the same and are omitted to conserve space.

The experiments show that inhibitor is destroyed in both acid and alkaline solutions. At hydrogen-ion concentrations between pH 5.5 and pH 8.7 the destruction is evidently much slower than in more acid or more alkaline solutions.

Ultrafiltration of Virus-inhibitor Mixtures. Although attempts to remove the inhibitor by dialysis failed, it seemed possible that the inhibitor particles were much smaller than the virus particles and that virus and inhibitor might be separated by ultrafiltration. To test the hypothesis, a virus-inhibitor mixture was prepared containing the infectious juice at a dilution of 1: 10 and inhibitor at a concentration of 16.2 mg. per cc. The mixture at pH 7.0 was filtered twice through Hy-flo Standard Super-cel. Ten cc. of the filtrate were then passed under 30-lb. pressure through an Elford membrane, prepared by Thornberry (31), with pores small enough to retain the virus. The precipitate on the membrane was washed by pass-

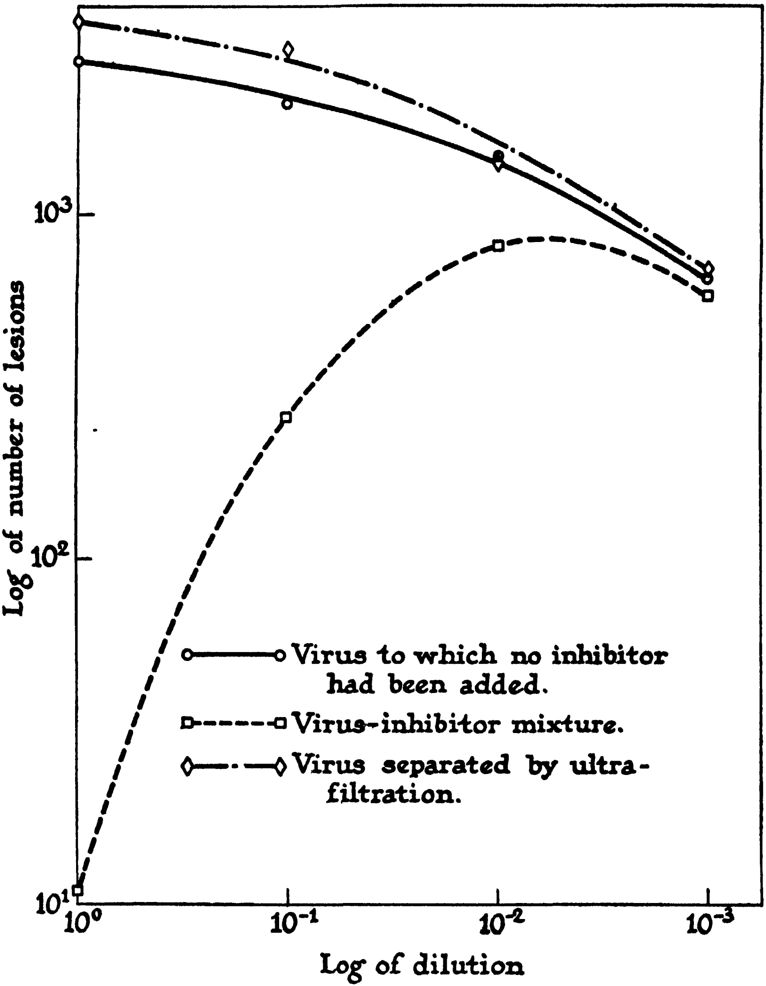

FIG. 5. Dilution curves showing separation of tobacco-mosaic virus and inhibitor by ultrafiltration.

ing 20 cc. of buffer through the membrane. The membrane and the precipitate were ground in 10 cc. of buffer, the coarse material removed by low-speed centrifugation, and the supernatant tested for infectivity at dilutions of 10^0, 10^{-1}, 10^{-2}, and 10^{-3}. For comparison, tests were made with the same dilutions of infectious juice without inhibitor and with the same dilutions of virus-inhibitor mixture, which had not been subjected to ultrafiltration. The lesions produced by each of the 12 solutions on 33 half-leaves are plotted in figure 5. The infectivity of the preparation that had been treated by ultrafiltration was as great as that of the inhibitor-free preparation, indicating that the virus had been freed from detectable amounts of inhibitor. These results were confirmed in a second experiment.

To determine if the inhibitor passed through the membrane, virus was added to the filtrate from the ultrafilter and the infectivity of the mixture tested on bean leaves. The filtrate reduced the infectivity of the virus, thus indicating that the inhibitor passed through the filter.

Ultracentrifugation of Virus-inhibitor Mixtures. It has been shown that the tobacco-mosaic virus can be sedimented by means of the ultracentrifuge (32). The probability that the inhibitor particles were smaller than the virus particles also suggested that a separation of the two might be effected by means of ultracentrifugation. A virus-inhibitor mixture was prepared containing a 10^{-1} dilution of infectious juice and an inhibitor concentration of 16.4 mg. per cc. Two 7-cc. samples of the mixture were ultracentrifuged for 1 hour in a field the mean of which was approximately 45,000 times gravity. The pellet from the first sample was resuspended in its own supernatant; that from the second was resuspended in 7 cc. of fresh buffer. The coarser particles in both samples were then removed by low-speed centrifugation. Each sample was ultracentrifuged again, the pellet resuspended as before, and the larger particles once more removed by low-speed centrifugation. The infectivities of 10^0, 10^{-1}, 10^{-2}, and 10^{-3} dilutions of the two samples were then tested on bean leaves along with corresponding dilutions of virus solution to which no inhibitor had been added. The number of lesions produced by each of the 12 solutions on 33 half-leaves is plotted in figure 6. The infectivity of the sample ultracentrifuged and resuspended in fresh buffer is as great as that of the virus solution alone, while the sample ultracentrifuged and resuspended in its own supernatant shows the dilution curve typical of virus-inhibitor mixtures. The data show that solutions can be freed of detectable amounts of inhibitor by ultracentrifugation. Two repetitions of the experiment gave similar results.

Possible Protein Nature of the Inhibitor. The inhibiting action of normal serum upon the infectivity of the tobacco-mosaic virus was noted by Mulvania (22). Since then the inhibitory action of protein solutions has been studied by a number of workers. The marked inhibitory action of trypsin has been especially studied (19, 8, 26, 9). Several workers (29, 1, 30, 2) have shown that enzymes like trypsin occur in insects. It may be that the inhibitor in insect juice is a trypsin-like substance. The nondialyzable, thermolabile nature of the inhibitor, its instability in acid or alkaline solutions, and the similarity of its action to that of proteins suggest that it may be a protein. With this possibility in mind, 3 stock inhibitor solutions were analyzed for protein nitrogen. The solutions analyzed were the same as those used in the experiments in which tests were made of the effect of various concentrations of inhibitor on the virus. The protein nitrogen was determined by a Kjeldahl micro-method described by Folin and Farmer (11) as modified by Northrop (23) but employing selenium oxychloride as a catalyst (18). The stock solutions with an inhibitor concentration of 17.94 mg. per cc. had a protein nitrogen content of from 0.16 to 0.18 mg. per cc. Calculations from data in figure 1 show that an inhibitor concentration causing a 50 per cent reduction in lesions would contain only about 0.0015 mg. of protein nitrogen per cc. However, only a fraction of the

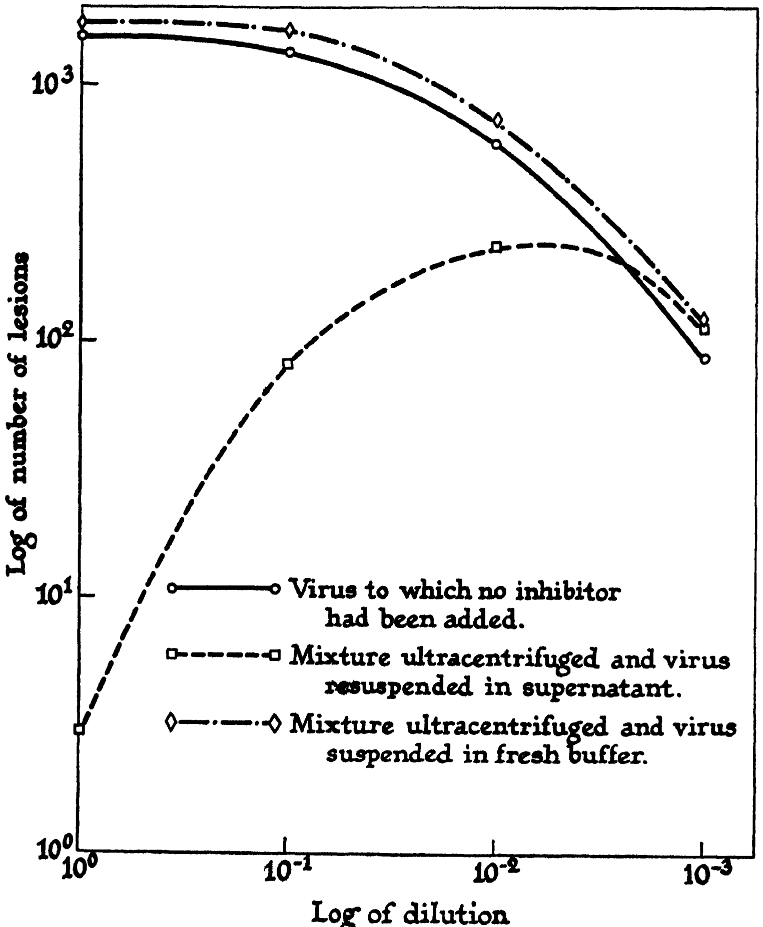

FIG. 6. Dilution curves showing separation of tobacco-mosaic virus and inhibitor by ultracentrifugation.

protein may be active. These results indicate that if the inhibitor is a protein it is a highly active one.

DISCUSSION

The apparently general inhibiting action of insect juices upon the infectivity of plant viruses would seem to be one reason for the practical absence of reports of successful direct inoculations of plants with juices of viruliferous insects and the failure to employ such inoculations for the study of plant viruses in their insect vectors. The success of McClintock and Smith (20) in inoculating spinach with the spinach-blight virus by pin punctures through juice from viruliferous aphids appears to be the one exception. Smith (24) reported successful inoculation of cowpeas with regurgitated juice or abdominal contents of bean leaf beetles, *Ceratoma trifurcata* Forst., that had fed on plants diseased with cowpea mosaic. This is hardly comparable with the problem under discussion, since his inoculum may not have been very different from freshly expressed juice from diseased plants. The successful transfer of virus to insects by feeding them juice of viruliferous insects (10) or by needle inoculation with the juice (28) gives no indication of the action of the inhibitor in such transfers. It may be that the inhibitor fails to act on the insect cells invaded by the virus.

Caldwell (9) holds that the inhibitory action of trypsin is brought about chiefly by an action upon the virus, while Stanley (26) holds that the principal action is upon the plant. The writer's experiments indicate that the chief action of the inhibitor in insect juices is not upon the virus. The injury of plants by concentrated solutions of inhibitor, its immediate action when added to virus solutions, and the fact that reduction in infectivity depends chiefly on inhibitor concentration and is affected only slightly by virus concentration support this conclusion. The experiments on the dilution, ultrafiltration, and ultracentrifugation of virus-inhibitor mixtures limit any important action between virus and inhibitor to an association of the two that is very readily broken without appreciable injury to the virus. However, the slight increase in the percentage reduction in lesions by a given concentration of inhibitor as virus concentration decreases suggests some action of the inhibitor on the virus. It is conceivable that insect juice might be quite destructive to a virus less stable than the tobacco-mosaic virus.

SUMMARY

Juices of the insect vectors *Aceratagallia sanguinolenta, Aedes aegypti, Aphis rumicis, Eutettix tenellus, Macrosiphum pisi, M. solanifolii, Macrosteles divisus,* and *Myzus persicae* inhibit the infectivity of tobacco-mosaic virus for Early Golden Cluster beans. Juices of macerated clover leaf hoppers inhibit the infectivity of plant juices containing the viruses of potato yellow dwarf, tobacco mosaic, potato X, turnip mosaic, tobacco necrosis, or tobacco ring spot No. 1, when mixtures of insect juice and virus are inoculated on suitable test plants.

The infectivity of certain mixtures of tobacco-mosaic virus and clover leaf-hopper juice can be increased by dilution or by heat treatment.

The inhibitor in clover leaf-hopper juice is thermolabile, not readily dialyzable, and unstable in acid or alkaline solution. If the inhibitor is a protein, as seems probable, it is very active, since 0.15 mg. of clover leaf hoppers, containing only about 0.0015 mg. of soluble protein nitrogen, reduces the infectivity of 1 cc. of a solution of tobacco-mosaic virus by 50 per cent. A given concentration of inhibitor in the presence of different concentrations of tobacco-mosaic virus reduces the number of primary lesions in beans by approximately a constant percentage.

Tobacco-mosaic virus is not destroyed by the inhibitor in clover leaf-hopper juice. The virus and the inhibitor can be separated by subjecting mixtures of the two to either ultrafiltration or ultracentrifugation.

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BROWN-ROT SCLEROTINIAS OCCURRING IN CALIFORNIA AND THEIR DISTRIBUTION ON STONE FRUITS[1]

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Brown rot, one of the important diseases of stone fruits in California, has long been a problem in the State, principally as a blossom- and twig-blighting disease, but occasionally as a severe fruit rot. This has been especially true in the coastal fruit-growing districts. In the interior valleys, particularly the peach-producing areas, brown rot has been considered an unimportant disease until within recent years, when losses of peach fruits from brown rot have become increasingly severe, amounting in 1936 to as much as 50 per cent of the fruit in some orchards.

The standard control measures now being used in California are spraying during the critical periods of infection and removing the mummied fruits and infected twigs where practicable. The latter practice minimizes the formation of sporodochia in the orchards during early spring. These control measures have been developed mostly for the control of brown rot of apricots and

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have been generally applied to other fruits. No importance has been attached to the perfect stage of the brown-rot fungus, and this phase was dismissed by Rudolph (20) with the following statement: "The apothecial stage is the great source of trouble in the eastern states, also in certain parts of Oregon. In California it is exceedingly rare and of no consequence in the propagation of the fungus."

The discovery in March, 1936, that apothecia were being produced in abundance from brown-rot-infected peach mummies in one of the important peach-growing sections of the State, and the subsequent proof of the pathogenicity of the organism suggested that there might be a difference in the etiology of brown rot in the various stone-fruit districts of California.

The investigations reported herein were planned to establish the identity of the causal organism or organisms and to determine their geographical distribution and host limitations within the State.

BROWN-ROT APOTHECIA FOUND IN CALIFORNIA

Apothecia resembling those of the brown-rot fungus were collected, apparently for the first time in California, by the junior writer on March 3, 1936, in the Sacramento Valley, Sutter County, under peach trees just coming into full bloom. Several sporulating peach mummies were found hanging in the trees at the same time, indicating the presence of brown rot in 1935. The apothecia were brought into the laboratory and photographed (Fig. 1, B, C, D), and thereafter induced to eject ascospores (13) onto potato-dextrose-agar plates and also into sterile distilled water. The ascospore suspension was atomized onto recently opened apricot blossoms, which were then placed in a saturated atmosphere in glass chambers at room temperatures. Control blossoms sprayed with distilled water were similarly incubated. After 2 days, the petals of all inoculated apricot blossoms showed symptoms typical of brown-rot blossom blight. After 4 days, moniliospores were abundantly produced on all infected blossoms, and the control blossoms remained healthy, except for advancing age. The agar plates seeded with ascospores showed abundant moniliospore production after 4 days' incubation at 25° C. (Fig. 1, E).

In April and May, 1936, several peach orchards in Sutter County, California, were visited by the authors and other members of the staff. Apothecia were found in the orchards in amounts varying from 1 to as many as 45 per orchard, and as many were found in orchards that had cover crops as in those that had very little vegetation.

Water suspensions of ascospores from each of 20 separate apothecia were sprayed upon branches of peach and nectarine blossoms and were incubated in moist chambers at room temperature. In all cases typical symptoms of blossom brown rot showed within 2 days, and an abundance of moniliospores developed within 4 days, whereas the control blossoms remained healthy.

Moniliospores from the ascospore cultures grown on potato-dextrose-agar plates and from blossoms previously sprayed with ascospores, after 2 days, produced typical symptoms of blossom brown rot when sprayed on peach, nectarine, and plum blossoms.

Five small branches each of almond, apricot, and peach were wounded by raising a portion of the bark with a scalpel, and were inoculated by inserting into the wounds small pieces of agar containing mycelium from the ascospore cultures. Three other branches, one of each kind of fruit, were used as controls and were treated in the same manner, except that sterile pieces of agar were used. All inoculated twigs developed cankers from 8 to 30 cm. long

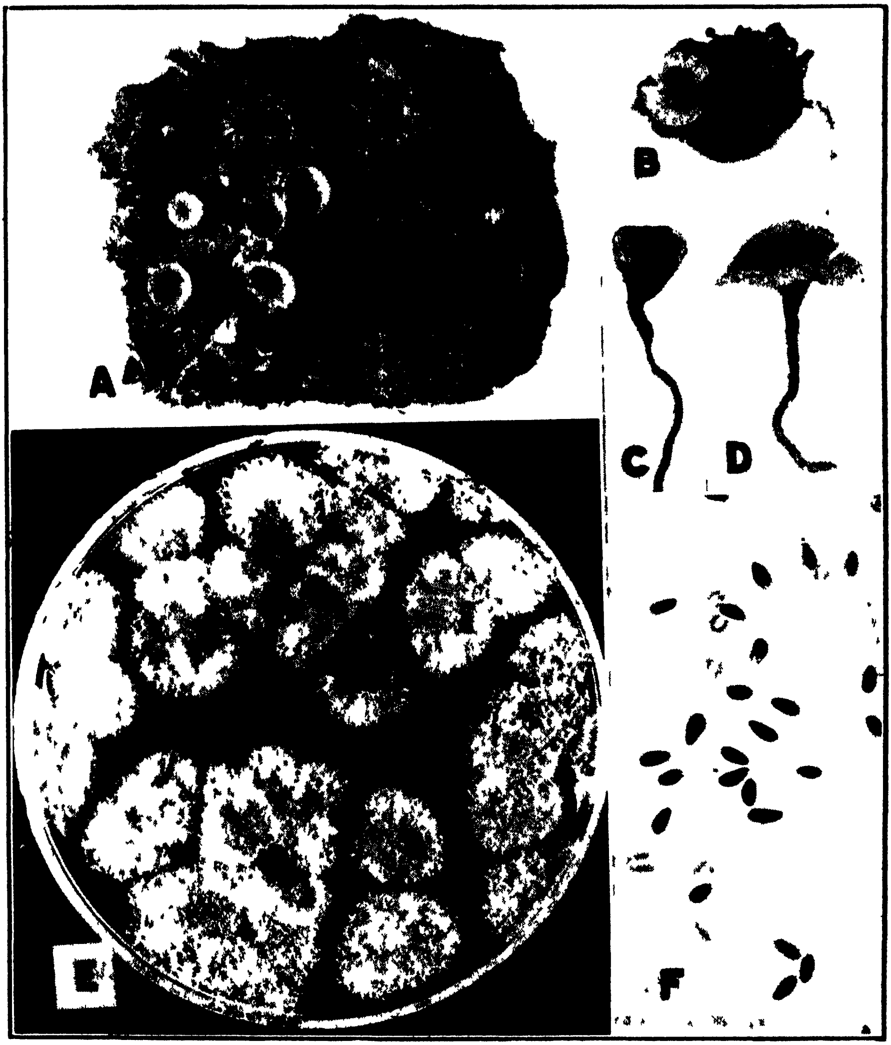

FIG. 1. A. Group of apothecia of *Sclerotinia fructicola*, on a single mummied peach. B, C, D. The first apothecia found in Sutter County, California, March 3, 1936. E. Culture of *S. fructicola* from ascospores discharged from apothecium D. F. Ascospores of *S. fructicola*.

within 14 days after inoculation, while none of the control branches developed any symptoms. Cultures of fungi similar to those inoculated into the branches were reisolated from the cankers.

The mature apothecia varied considerably in size. The length of the stipe varied with the specimens and the distance of the mummy from the surface of the ground. The stipe of one apothecium measured 6.75 cm. A number of the apothecia developed from mummies that were completely buried under the surface soil. The color of the apothecia ranged from apricot to light brown and darkened considerably with age.

The measurements of asci and ascospores from the apothecia found in California (Table 1) are within the same range of measurements as reported by Bartram (2), Valleau (23), Matheny (14), Pollock (16), Jehle (12), Roberts and Dunegan (17, 18, 19), and Harrison (7, 8). A photomicrograph of ascospores from one of the apothecia is shown in figure 1, F.

The cultures obtained from ascospores were compared with other cultures isolated from blighted apricot twigs gathered at the University of California, College of Agriculture, Experiment Station orchard, near Davis. The cultures from the 2 sources were found to be distinctly different in gross characteristics when grown on either potato-dextrose agar or prune-juice agar (Fig. 2, A, C), indicating that possibly 2 separate organisms were represented.

Moniliospores of the organisms from the 2 sources were measured, and the range and the means of widths and lengths calculated and recorded in table 1. The culture from ascospores from a single apothecium used in these measurements is referred to in table 1 as culture No. 48–6, and the culture isolated from blighted apricot twigs as culture No. 49–2. The mean measurements of moniliospores produced on peach blossoms inoculated with ascospores (culture 48–6) were 10.46 × 16.24 μ; and of moniliospores produced on potato-dextrose agar seeded with ascospores from the same apothecium (culture 48–6) were 9.09 × 14.24 μ. In comparison the mean measurements of moniliospores of culture 49–2 produced on potato-dextrose agar were 8.02 × 12.00 μ. The size of the moniliospores apparently depends somewhat upon the medium on which the fungus is grown, and the range in width and length of moniliospores of both cultures 48–6 and 49–2 overlaps considerably. Differences between the mean measurements of moniliospores of cultures 48–6 and 49–2 probably are significant; but, in the same way, mean measurements of 2 different lots of spores from the same sources showed significant differences. It is, therefore, unlikely that the size of moniliospores alone could be depended upon to separate the two organisms. This is in agreement with the findings of Harrison (8, 9), Ezekiel (5), and others who have considered moniliospore measurements as a possible means of separating species of brown-rot fungi.

The moniliospores of both types were produced in chains with no disjunctors. Those of culture 48–6 germinated with a relatively long straight germ tube before branching. Germinating moniliospores from culture 49–2 produced a relatively short, irregular germ tube, and then branched freely (17).

The pathogenicity of the two types of organisms was compared on cherry

TABLE 1.—*Measurements of asci, ascospores, and moniliospores of Sclerotinia fructicola and moniliospores of S. laxa*

Sclerotinia species	Form measured	Source	Width			Length		
			Range	Mean	Standard error of mean	Range	Mean	Standard error of mean
S. fructicola	Asci	Apothecia fresh mounts	7.48 to 12.21	9.68	0.124	119.41 to 193.62	160.27	1.31
S. fructicola	Ascospores	Apothecia fresh mounts	4.23 to 7.64	5.40	0.038	7.48 to 13.89	10.66	0.070
S. fructicola, Culture 48–6	Moniliospores	Peach blossom ascospore inoculation	7.64 to 14.35	10.46	0.065	12.21 to 21.22	16.24	0.097
S. fructicola, Culture 48–6	Moniliospores	Ascospore culture on potato-dextrose agar	7.33 to 11.61	9.09	0.083	10.84 to 18.02	14.24	0.114
S. laxa, Culture 49–2	Moniliospores	Isolation from apricot twig blight on potato-dextrose agar	6.27 to 10.23	8.02	0.049	9.47 to 15.27	12.00	0.069

fruit by atomizing the fruits in moist chambers with a water suspension of conidia from potato-dextrose-agar plates. Culture 48–6, originally obtained from the ascospores, rotted the fruit readily and produced an abundance of conidial pustules on the surface of the cherries 3 days after inoculation; whereas culture 49–2, originating from apricot twigs, produced only small lesions and no spores at the end of 3 days (Fig. 2, B, D). Similar results were obtained on peaches and apricot fruits.

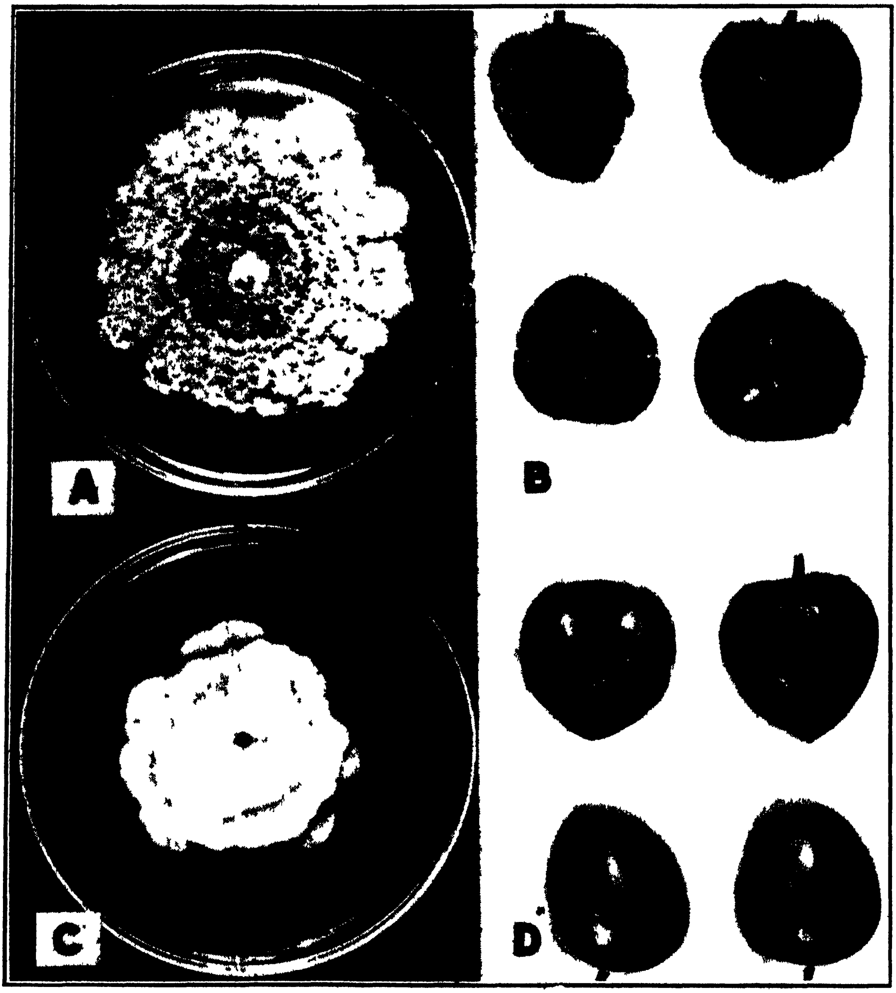

FIG. 2. A. Seven-day-old culture of *S. fructicola* on potato-dextrose agar. B. Cherry fruits, variety Grant, 3 days after inoculation with a water suspension of moniliospores of *S. fructicola*. Almost all of the fruits became infected and produced an abundance of moniliospores on the surface. C. Seven-day-old culture of *S. laxa* on potato-dextrose agar. D. Cherry fruits, variety Grant, 3 days after inoculation with a water suspension of moniliospores of *S. laxa*. Only small lesions developed and moniliospores were not yet evident.

The cultures were compared with previously published descriptions of brown-rot fungi by Norton (15), Wormald (25, 26, 29), Barss (1), Ezekiel (5, 6), Roberts and Dunegan (17, 19), and Harrison (7, 8, 9, 10). Culture 48–6, from ascospores discharged from apothecia found developing from mummied peaches, was determined to be *Sclerotinia fructicola* (Wint.) Rehm. Culture 49–2, isolated from blighted apricot twig at Davis, was found to be *Sclerotinia laxa* Ader. and Ruh. This conclusion was verified by T. H. Harrison who, after examining the cultures, wrote as follows: "While time has not permitted an exhaustive study of the position, I am sure that you were right in your original designation of the species concerned. Culture No. 49–2 is behaving as a typical *S. laxa* culture, while cultures No. 48–6 and No. 48–2 are typical of *S. fructicola.*"

CHARACTERISTICS AND EARLY ACCOUNTS OF SCLEROTINIA LAXA AND S. FRUCTICOLA IN CALIFORNIA

The finding of apothecia of *Sclerotinia fructicola* in abundance in the peach orchards of the Sacramento Valley, and the fact that both *S. fructicola* and *S. laxa* are involved in the brown rot of fruits within the State, emphasized the need for more information concerning the etiology of brown rot in the stone-fruit sections of California.

Apparently, a thorough study of the species of fungi causing brown rot of fruits in California has not been made. Earlier investigators of the disease in the State have been concerned primarily with the cycle of development of the blossom- and twig-blight stages and measures for their control.

Species designations, such as *Sclerotinia americana, S. cinerea, S. fructicola,* etc., have been applied to the brown-rot fungi of California without critical studies as to their identity, and, apparently, on the assumption that the fungus was the same as that described in other parts of the United States. The names applied to the brown-rot fungi in the earlier California publications concerning the disease cannot, therefore, be relied upon as being entirely descriptive of the species. There are, however, certain features characteristic of *S. laxa* and others characteristic of *S. fructicola* that make it possible when reading these earlier publications to distinguish with considerable accuracy the species of *Sclerotinia* with which the publication deals.

Distinguishing Characteristics. Sclerotinia laxa Ader. and Ruh. produces an abundance of sporodochia on overwintering blighted twigs and cankers (Fig. 3). In California they have been found in abundance on blighted twigs of almond, apricots, cherries, plums, peaches, nectarines, and flowering quince, *Cydonia japonica.* On the other hand, as pointed out by Brooks and Fisher (4), Smith (21), and by Roberts and Dunegan (19), *Sclerotinia fructicola* rarely produces conidial tufts on overwintering blighted twigs and cankers. Furthermore, in these studies during the past 2 seasons, isolations have been made from overwintering blighted twigs and cankers of almond, apricot, cherry, peach, nectarine, and plum, and in no case has *S. fructicola,* isolated from those twigs and cankers, been found producing conidial tufts. Both fungi, however, have been repeatedly isolated from the sporodochia on old mummied fruits that had hung in the trees over winter.

Similarly, according to Barss (1), Ezekiel (6), and Roberts and Dunegan (19), *Sclerotinia laxa* is principally a blossom- and twig-blighting fungus; though, under certain conditions, it may cause considerable fruit rotting; whereas *S. fructicola* is stated to be mostly a fruit-rotting fungus, though it does also cause some twig blight.

There is no evidence that apothecia of *Sclerotinia laxa* have ever been found in the United States, while, in most of the localities where *S. fructicola* has been reported, an abundance of apothecia of the fungus has been found developing from mummied fruits (8, 10).

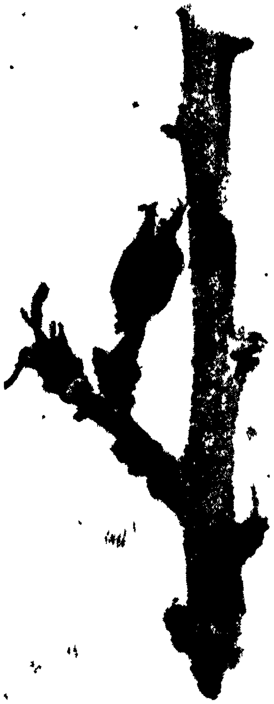

FIG. 3. Blighted almond twig infected with *Sclerotinia laxa* showing sporodochia. × 2.

Early History of Brown-Rot Fungi in California. The earliest account of brown rot of fruits occurring in California was published by Bioletti (3) in 1900. He described the rotting of apricot fruits in the orchards of the San Francisco Bay counties and also mentioned the presence of the disease on plums. Although he did not describe blossom and twig blighting, he mentioned this stage of the disease. Later, Smith (22), referring to the brown rot in the same districts, pointed out the damage to fruit trees by blossom and twig blighting and also that fruit rotting was periodic, depending on climaic conditions. Other reports of the disease, mostly in experiment-station reports, refer to its spread and to the relative amount of damage.

Howard and Horne (11), in 1921, working with brown rot in Santa Clara County, published descriptions of the sporodochia on overwintering blighted twigs and cankers, thus indicating that they were concerned with *Sclerotinia laxa*. They also stressed the importance of blossom and twig blighting in the apricot orchards of the bay area.

In addition to this, Wormald (27) reports having received a mummied apricot, a peach fruit, and a blighted apricot twig in 1920 from W. L. Howard, of the California Agricultural Experiment Station. From these specimens fungi were isolated, which he states were identical with *Sclerotinia cinerea* = *S. laxa* of Europe.

Ezekiel (5) was probably the first to publish an account of a comparative study of brown-rot fungi gathered from different parts of the world, which included isolations from California (Table 2).

TABLE 2.—*The species of brown-rot Sclerotinia isolated by Ezekiel from material he obtained from California in 1923*

Culture number	Host	Name given isolations by Ezekiel, 1924	Name applied to the fungus at present
S45[a]	Apricot	*S. americana*	*S. fructicola*
S46	Peach	*S. cinerea* f. *pruni*	*S. laxa*
S47	Peach	*S. cinerea* f. *pruni*	*S. laxa*
S49	Cherry	*S. cinerea* f. *pruni*	*S. laxa*

[a] The specimens from which the isolations were made were obtained from B. A. Rudolph, of the California Agricultural Experiment Station. In a recent communication from Dr. Rudolph, he states that the specimens probably came from Santa Clara County.

Thus, Ezekiel reported the identification of 3 cultures of *Sclerotinia cinerea* = *S. laxa*, S46, S47, and S49, whereas culture S45 he believed to be *S. americana* = *S. fructicola*. This, as far as evidence is available, seems to be the first and only authentic account, based on cultural studies, of the occurrence of *S. fructicola* in California. Earlier writers who assumed this species to be present in California apparently did so without presenting evidence other than the earlier written accounts, which inadequately describe the species.

Rudolph (20), working in California, principally on the problem of controlling brown rot in apricots, emphasized the importance of blossom and twig blighting. He clearly described the manner in which sporodochia are produced on overwintering blighted twigs, and presented pictures of them on blighted apricot twigs. He mentioned the problem of fruit rotting, but dismissed this phase of the disease by stating that it was to be discussed in a later publication.

The fact that Ezekiel (5) described both *Sclerotinia laxa* and *S. fructicola* from specimens obtained from Rudolph shows that both species were present in California at that time. It is probable, though, that most of the blossom and twig blighting of apricots was due to *S. laxa*. The fruit rotting may have been caused by either or both species. If *S. fructicola*, however, had been abundant in the orchards of California, those working with the disease undoubtedly would have found the apothecia developing from old mummies in the orchard soils. Apparently, none were found, as there are no previous records of anyone finding apothecia of either *S. fructicola* or *S. laxa* in the State.

Rudolph (20) stated that the apothecial stage of the brown-rot fungus was exceedingly rare in California and of no consequence in its propagation. Recently, in correspondence with the writers, he stated that no instance of apothecial formation was known to him at that time.

In a publication entitled "Almond Culture in California" by Milo N. Wood (24), E. E. Wilson, who wrote the section on diseases, reports that *Sclerotinia fructicola* causes the brown rot of almonds in California. He shows a picture of blighted twigs upon which groups of sporodochia had developed, indicating that he was dealing with *S. laxa* rather than *S. fructicola.*

In a recent survey of the brown-rot fungi occurring in the almond orchards throughout the State, the writers have isolated only *Sclerotinia laxa* from blighted blossom twigs and old cankers of the almonds.

Wormald (29) reports that *Sclerotinia laxa* and *S. fructicola* infect the apricot in California. It seems probable that he obtained this information from the work of Ezekiel.

The above evidence indicates that during the earlier history of the disease in the State *Sclerotinia laxa* was the predominating species causing brown rot of stone fruits in California, particularly within the San Francisco Bay counties where most of the research on the disease has been conducted.

SURVEY OF CALIFORNIA ORCHARDS FOR BROWN ROT

A survey of the principal stone-fruit districts of California has been made in order to collect the species of *Sclerotinia* causing brown rot in the State and to determine their distribution.

Cultures of the brown-rot fungi were obtained by making tissue plantings from blighted twigs, cankers, mummies, rotting fruit, and also from conidial tufts on mummies and blighted twigs.

The species of *Sclerotinia* isolated in this survey were identified by their growth characteristics in culture, based on the work of Wormald (25, 26), Barss (1), Ezekiel (5, 6), Roberts and Dunegan (17, 19), and Harrison (7, 8), who have shown that the brown-rot *Sclerotinias* may be separated in this manner. In general, the distinguishing characteristics used to separate *Sclerotinia fructicola* and *S. laxa*, when grown on potato-dextrose agar, are briefly as follows:

Sclerotinia fructicola grows rapidly; the colonies are mostly entire and spread evenly over the plate; the conidia are produced in abundance, are mostly confluent, and frequently develop in concentric rings (Fig. 2A).

Sclerotinia laxa grows relatively slowly; the colonies are characteristically lobed, grow in concentric zones, are more closely appressed to the surface, and produce considerable sub-surface mycelium; the conidia are produced in relatively few scattered tufts over the surface of the colony (Fig. 2C).

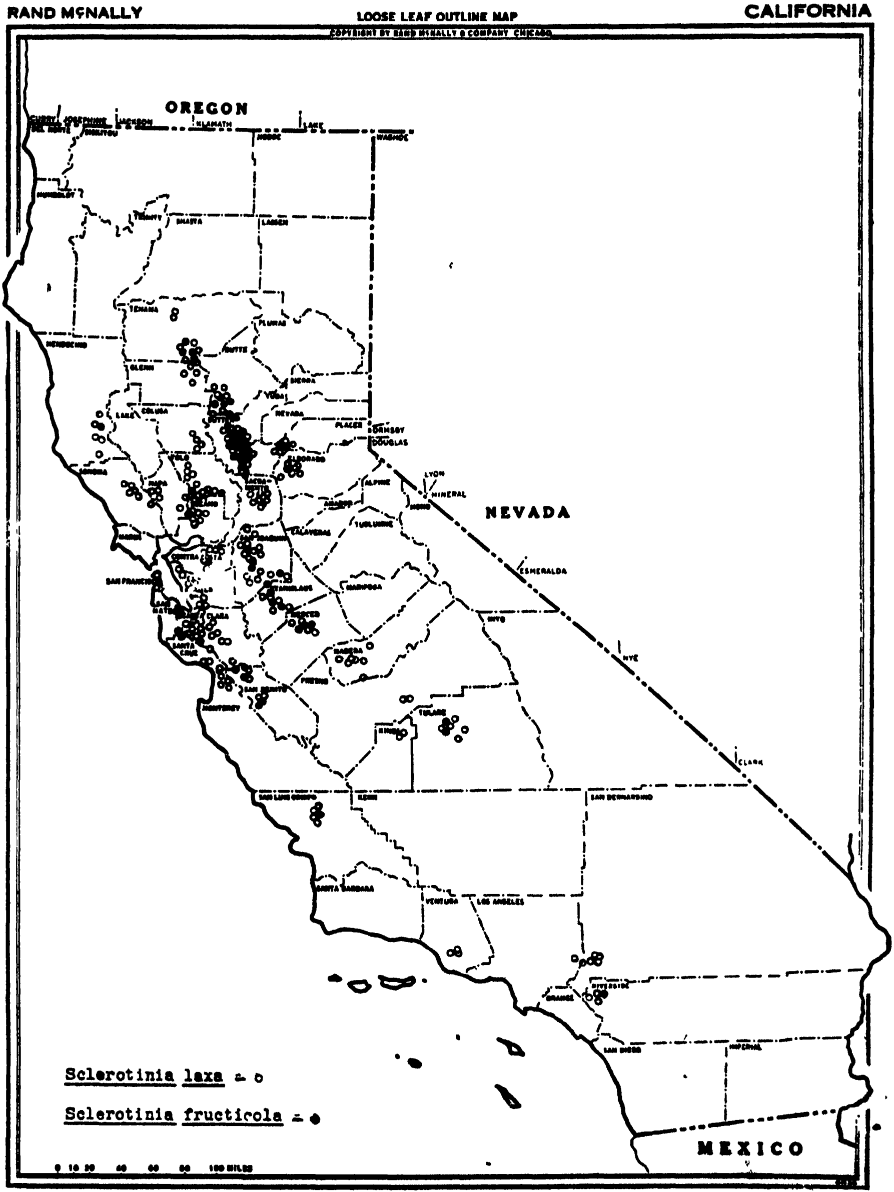

FIG. 4. An outline map of California and the counties showing the distribution of *Sclerotinia fructicola* and *S. laxa* on stone fruits. The dots indicate the approximate location where *S. fructicola* was collected and the circles indicate locations where *S. laxa* was collected. Map published with permission of Rand McNally and Co. under License No. 3802.

Although a number of variations of the 2 species were found, the characteristics are such that most of the forms isolated were identified without difficulty, and no forms have been found that may be designated as new species purely on the basis of cultural characteristics.

From 1 to as many as 25 isolations have been made from 178 separate orchards scattered throughout the fruit-growing districts The results of these isolations showing the distribution of *Sclerotinia laxa* and *S. fructicola* in the State are shown in figure 4.

Sclerotinia laxa was found in abundance through all of the San Francisco Bay counties, where brown rot was first reported in the State, and where the blossom- and twig-blighting phase of the disease has been most serious. In this region *S. laxa* was isolated from flowers, blighted twigs, and fruits of apricot, prunes, nectarines, peaches, cherries, varieties of flowering peach and flowering cherries, blighted blossoms and twigs of almonds, Wickson and Tomentosa plums. *S. fructicola,* however, was found in 6 localities in the San Francisco Bay region as follows: (1) A single culture from the fruit of flowering peach collected near Berkeley in Alameda County; (2) a single culture from a nectarine fruit from Contra Costa County; (3) several cherry fruits collected in an orchard in Santa Cruz County near Santa Cruz; (4) several apricot fruits collected from 2 nearby orchards in San Benito County; (5) several cultures from blighted peach twigs and mummied peach fruits collected near Palo Alto, Santa Clara County; and (6) several cultures from mummied peach fruits from a peach orchard in San Mateo County near Palo Alto. In addition, Ezekiel's culture S45 (5) from apricot, which he reports as *S. americana* = *S. fructicola,* was obtained from B. A. Rudolph and probably originated in Santa Clara County. All other isolations from recorded collections made in the San Francisco Bay counties have yielded *S. laxa.*

Isolations from specimens collected in the San Francisco Bay region show that *Sclerotinia laxa* is widespread on all of the stone fruits in the district and that *S. fructicola,* while present, is not abundant. The latter appears to have entered the region within recent years; or, if it has been there for sometime, it has failed to become well established.

In the large peach-producing areas of the upper Sacramento Valley, both *Sclerotinia laxa* and *S. fructicola* have been isolated. Tissue plantings from most of the rotting and mummied peach fruits gathered in this area have yielded *S. fructicola.* Both *S. fructicola* and *S. laxa* have been isolated from blighted blossoms and twigs of peach, nectarine, apricot, and cherries. *S. laxa* has been the only brown-rot organism isolated from blighted blossoms and twigs of almonds.

Isolations from stone fruits from the San Joaquin Valley, including San Joaquin, Merced, Madera, Fresno, Kings, Tulare, and Kern counties, have yielded both *Sclerotinia fructicola* and *S. laxa.* Most of the cultures of *S. fructicola* have come from peaches and nectarines, whereas *S. laxa* was isolated from almond, peach, nectarine, and plums.

Only a few collections have been made from the southernmost portion of the State. One culture of *Sclerotinia fructicola* was obtained from a mummied plum from Riverside County, whereas all other isolations from southern California have yielded *S. laxa.*

During the course of this survey, *Sclerotinia laxa* has been isolated from stone fruits in every section of the State from which specimens of brown rot have been collected. It has caused an abundance of blossom blighting, followed by twig blighting, in all of the stone fruits from which specimens have been taken, and it has caused some fruit rotting in most of them. On the other hand, incidence of *S. fructicola* was not so general throughout the fruit districts, but was more localized. It was abundant in the large peach-producing sections of the Sacramento and San Joaquin valleys and was only occasionally observed in the apricot- and prune-producing sections of the coastal valleys. *S. fructicola* was frequently isolated from fruits, and occasionally from flowers and twigs, of apricots and plums grown adjacent to *S. fructicola*-infested peach orchards in the Sacramento Valley. It has not, however, been recovered from almonds under the same conditions; whereas, in these same almond orchards, *S. laxa* frequently was abundant. *Sclerotinia fructicola* was much more destructive to fruits than *S. laxa,* but caused less severe blossom and twig blighting.

DISCUSSION

The opinions of growers and other observers that the brown-rot problem in the peach-producing areas of the Sacramento Valley has changed within recent years from a mild infestation, consisting chiefly of blossom and twig blighting, to a destructive form characterized by severe fruit rotting may be explained in part if we assume that *Sclerotinia fructicola* has been introduced into the area comparatively recently. Although *S. fructicola* is distributed through most of the peach-producing sections of the State, it is much more localized than is *S. laxa* and has not been found in the peach and nectarine plantings of Yolo, Solano, and Napa counties. The previous failure of investigators to find apothecia may be taken as additional evidence of recent introduction.

The knowledge that apothecia are abundantly produced may necessitate changes in the control practices for the peach-growing areas, but current information is insufficient to justify definite recommendations.

SUMMARY

Brown rot of stone fruits for a number of years has been serious as a blossom- and twig-blighting disease and occasionally has caused considerable fruit rot in the San Francisco Bay counties of California. Within recent years, however, it has appeared in the peach orchards of the interior valleys, mostly as a fruit rot.

Apothecia, herein shown to be those of *Sclerotinia fructicola* were found in California on March 3, 1936, apparently for the first time. They were observed on mummied peach fruits in an orchard in the Sacramento Valley and have since been found in a number of peach orchards in the same region.

Sclerotinia laxa was isolated subsequently from diseased apricot twigs, and the identity of the 2 organisms was determined by comparisons in culture, spore measurements, pathogenicity studies, and comparison with previously published descriptions.

A discussion of the earlier studies of brown rot relative to the species of *Sclerotinia* causing the disease of stone fruits in California is presented. It shows that *Sclerotinia laxa* has long been present there and probably has been responsible for most of the brown rot described in these earlier publications, and that *S. fructicola* may have been introduced into the State relatively recently.

The results of a brown-rot survey of the principal stone-fruit districts of California are presented, showing the distribution of *Sclerotinia laxa* and *S. fructicola.* The former was found throughout all of the stone-fruit districts from which specimens were obtained, whereas the latter was more localized, occurring most abundantly in the peach-producing areas of the Sacramento Valley.

In localities where *Sclerotinia fructicola* occurred, it was isolated most frequently from brown-rot-diseased fruits, whereas *S. laxa* was isolated more frequently from blighted blossoms and twigs.

In no instance has *Sclerotinia fructicola* been isolated from overwintered blighted twigs of stone fruits that were producing sporodochia. All isolations from these twigs have yielded *S. laxa.*

Sclerotinia laxa was the only organism isolated from brown-rot-affected flowers and twigs of almonds collected during this survey.

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AVENUES OF ENTRANCE FOR CANKER-FORMING NECTRIAS OF NEW ENGLAND HARDWOODS

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INTRODUCTION

A general history of the Nectria-canker disease of broadleaved trees, which, by various writers is called "canker," "European canker," and "Nectria canker," is given by Zeller (10) and Ashcroft (1). Accounts of Nectria canker in New England are given by Welch (7) and Spaulding, Grant, and Ayers (6). In this paper, which presents field and inoculation data on avenues of entrance for the canker-forming *Nectrias,* reference to previous publications is largely limited to work directly bearing on this subject.

Hartig (3), several years before 1880, noted that infections in the bark usually were preceded by injury. He found that injuries in the cortex of the axils of lateral branches were infection courts. Hail, frost, freezing, and insects were considered as common causes of injuries through which infection took place.

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[1] The writers wish to express their appreciation to Dr. T. W. Childs for assistance in collecting field data and to Mrs. A. J. Watson for helping with the culture work.

Wiltshire (8), in studies of the canker fungus (*Nectria*) on apple, pointed out that a most important path of infection was through the scars left by fallen leaves. He (9) also described the infection of apple trees by the canker fungus through scab (*Venturia inaequalis* (Cke.) Wint.) lesions. Zeller (10), in studying the European canker of pomaceous fruit trees in Oregon, found that the fungus gained entrance into a tree by means of unprotected wounds and small injuries, or through such natural structures of the host as leaf scars. He also noted infections through lesions produced by winter injury, *Monilia* sp., and *Botrytis cinerea* Pers. In writing about infection through crotch, frost, and other cracks in the bark, he referred to a number of European workers (Hartig, Münch, Lapine, Goethe, Cotton) who mentioned the occurrence of cankers arising from infections through cracks at the crotches of branches, but apparently no one had inoculated the axillary cracks.

Ashcroft (1), in describing the "European canker" on black walnut, noted the frequent occurrence of a lateral branch stub in the center of cankers. Jackson[2] stated that the constant association of canker centers and branch bases points strongly to the fact that most trunk infections are probably a continuation of branch and twig infections. Andrews,[3] reporting the results of a survey of Nectria canker near Kane, Pennsylvania, found that the points of entry of approximately 67 per cent of the infections were dead branch stubs, 23 per cent were open wounds or bark injuries, and 10 per cent could not be determined. Kienholz and Bidwell (5), in summarizing the disease-survey data for Connecticut forests, showed that "Many Nectria cankers were associated with old branch stubs as a possible avenue of entrance while others were associated with borer injury, frost cracks, rubs and mechanical injuries. The avenue of entrance could not be determined in many cases." Hepting (4), in a brief general summary of the then available knowledge of the canker Nectria of hardwoods, mentioned that, except in basswood and possibly yellow poplar, which it may enter through any bark injury, this fungus usually enters through a small, dead branch stub. From this point it spreads into the bark of the trunk forming the cankers. Often the stub through which the fungus entered can be found in the center of the canker.

AVENUES OF ENTRANCE FOR NATURALLY FORMED NECTRIA CANKERS

A study of naturally formed Nectria cankers on various hardwoods was made in State and National forests of Connecticut, Vermont, and New Hampshire, with reference to the avenues of infection (2). The cankers studied varied in size and age and location on the hosts. The results are summarized in table 1. Of the 3,161 cankers examined, 29 per cent had measured dead branch stubs at the apparent center of canker development,

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[2] Jackson, L. W. R. Nectria canker on yellow birch (*Betula lutea*) in Pennsylvania. Mss. report, 4 pp. May 26, 1933.

[3] Andrews, S. R. The incidence of Nectria canker in the sugar maple-beech-yellow birch type near Kane, Pennsylvania. Yale Forest School, M. F. thesis, Mss. 1935.

TABLE 1 —*Avenues of infection on naturally formed Nectria cankers on different hosts*

Host group	Number of cankers														
	With measured central dead-branch stub of indicated diameter (inches)						With un-measured central dead-branch remnants	In axils of living branches of indicated diameter (inches)						In rubbing injuries	With undeter-minable avenue of infec-tion	Total
	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{1}{2}$	$\frac{3}{4}$	1	Total		$\frac{1}{4}$	$\frac{1}{2}$	1	2	3	Total			
Birches	230	160	91	17	22	520	340	7	42	88	45	20	202	73	231	1366
Maples	34	88	74	4	12	212	277	12	57	80	37	20	206	140	297	1132
Aspens	18	52	37	6	8	121	137	1	2	4	2	1	10	8	104	380
Oaks Hickories Pin cherry	11	27	26	4	4	72	89	2	13	31	3	3	52	24	46	283
Total	293	327	228	31	46	925	843	22	114	203	87	44	470	245	678	3161

27 per cent had unmeasured dead branch remnants at the center, 15 per cent were formed in the axils of living branches, 8 per cent were found in conjunction with injuries caused by rubbing, and in 21 per cent it was impossible to determine the avenue of entrance. These data show that branches frequently are involved in the early stages of canker formation. Data on the diameters of the measured dead-branch stubs found in the centers of cankers and on the diameters of living branches having axillary or crotch cankers are grouped by diameter classes in table 1. The diameters of 92 per cent of the 925 dead-branch stubs were from ⅛ to ½ inch, and the diameters of 95 per cent of the 470 living branches having axillary cankers were from ½ to 3 inches. Twenty-five per cent of all of the cankers with measured dead-branch stubs and 24 per cent of all of the axillary cankers fell in the ½-inch diameter classes.

These data suggest that when a branch of ½-inch diameter becomes infected at its axil with the Nectria-canker fungus, chances are about equal that it may be girdled or may live and the canker become axillary. If the branch infected near or at its axil is less than ½ inch in diameter, the fungus is apt to girdle and kill the branch, which later breaks off, leaving only a dead stub as evidence of the avenue of infection. When the fungus enters a crack in the axil of a branch more than ½ inch in diameter, it is more apt to form a crotch or axillary canker and the branch is apt to remain alive.

The cankers classed as with branches less than ½ inch in diameter include some in which the canker centers were occupied by dead short spurs or buds. Such cankers were found most often on the birches. It was observed that branches over ½ inch in diameter sometimes bore one or several localized cankers that had smaller branch parts in their centers. It was apparent that for branches more than ½ inch in diameter to serve as avenues of entrance for Nectrias into larger branches or trunks, infection had to occur at or very close to their axils.

RESULTS OF INOCULATIONS

In September, 1935, wound (knife-incision) inoculations using mycelial cultures of canker *Nectria* on potato-dextrose agar were made close to the axils of 25 living and 5 dead branches. The checks consisted of plain agar placed in similar wounds at the axils of 10 living branches. The test was made at Black Brook Trail, Carroll, New Hampshire, and the species inoculated were red maple[4] and yellow birch. Up to October, 1937, none of the inoculations at the axils of dead branches had resulted in canker formation or development of *Nectria* perithecia. Likewise, no *Nectria* fruiting or canker formation had occurred on the checks, although 1 branch of the 10 died from an undetermined cause. On the other hand, of the 25 living branches inoculated at the axils, 15 were girdled and dead with Nectria perithecia present on the bark near the inoculation. Three branches were dead, with no *Nectria* perithecia present. Five branches remained alive and were cankered with *Nectria* perithecia present on the surface of the dead bark within the cankered area. On 2 branches small lesions were produced, but no fungus fruiting was evident and the branches remained alive. Reisolations showed that the *Nectria* fruiting on the successful inoculations was the same as that used for the inoculum. These results confirm observations and experience that lead the writers to the opinion that the canker *Nectrias* enter trees through living or dying (but not dead) tissues. Entry is made through living branches, which may die before the fungus fruits, but not through branches already dead from some other cause.

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[4] Common names of trees used by the authors are according to G. B. Sudworth's Check list of the forest [illegible] of the United States, their names and ranges. U. S. Dept. Agric. Misc. Circ. 92. [illegible] pp. 1927.

Figure 1, A and B, shows canker formation on branch and trunk resulting from inoculation with mycelial cultures in injuries made close to the axils of the branches. Figure 1, A, shows the results of inoculating a living branch of red maple $\frac{1}{2}$ inch from the crotch. The branch was $\frac{3}{8}$ of an inch in diameter when inoculated. In September, 1936, it was dead and bore *Nectria* perithecia on the bark surrounding the inoculation. This fungus was the same species as that used for the inoculations. The photograph taken in May, 1937, shows splitting of the bark on the trunk and, also, groups of perithecia. The infection had extended into the trunk from the inoculation. Figure 1, B, shows a similar spread of infection into the trunk

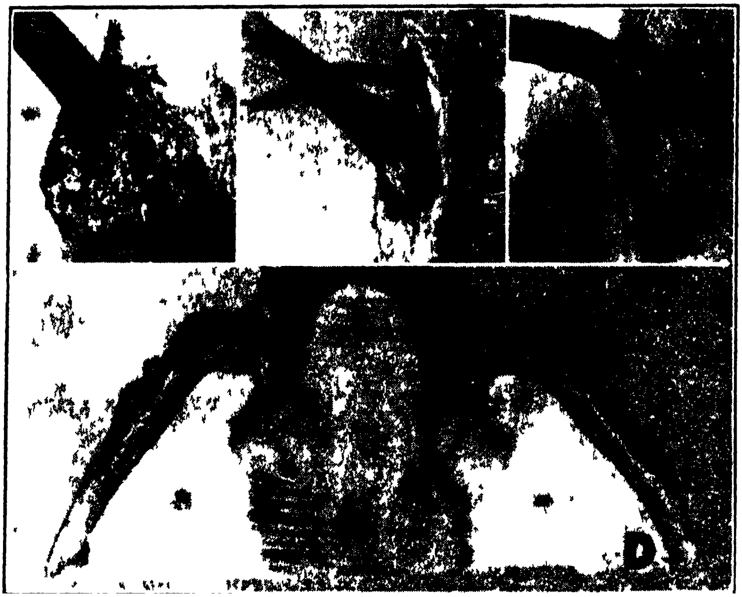

FIG. 1. Condition of typical branches about one and a half years after inoculation at branch axils with mycelial cultures of *Nectria*. A. Canker extending from branch into trunk of red maple. Note presence of perithecia on bark of branch and trunk. B. Peeled canker extending into trunk of yellow birch. C. Branch of red maple girdled by fungus with no visible extension of infection to trunk. D. Cross section of infected branch and trunk of red maple. Note that the roll of apparently healthy callus was pushing out the old infected bark.

of a yellow birch from an inoculation on a living branch. The bark was removed to show clearly the girdled branch, the development of 1936 annual growth on the branch beyond the infected area, and the extension of the canker onto the trunk. Although the branch was completely girdled at the base by the fungus, the distal end continued to live and formed an annual ring of wood in 1936, but it was entirely dead in May, 1937.

Spread of the fungus into the trunk did not occur in all branch inoculations. Figure 1, C, shows the infection resulting on a red maple branch as a small, slightly sunken area around the point of inoculation. The branch was dead in September, 1936, and *Nectria* perithecia had formed on the bark of the branch in the infected area. Here, a roll of callus had formed on the trunk at the axil of the branch, and progress of the fungus appears to have been checked by it. A cross-section cut from another inoculation on the same tree and similar in external appearance is shown in figure 1, D. Here, infection extended into the trunk, but the roll of apparently healthy callus was pushing out the old infected bark. Within the bark on the left note the light-colored line extending from the edge of the callus roll and separating the living from the dead bark. Staining with Sudan IV turned this line red, indicating that it is suberin or corky in nature. The normally formed outer layer of the bark gave the same red reaction and was continuous with the deeper seated and more recently formed red-stained layer. Careful examination showed that other suberized (red-stained) layers had been formed, but apparently had not been successful in checking progress of the fungus. Why some of these layers had not prevented the spread of the fungus or whether the last-formed layer would successfully check it could not be determined.

Zeller (10), working with pomaceous fruit trees, found that leaf-scar inoculations made in late spring sometimes produced lesions. These lesions, however, were superficial scales of diseased-bark tissue and were sloughed off early, as the host at that time of year formed a cork cambium in the inner layers of the parenchyma of the pericycle. This cork cambium was continuous with that of the outer bark in the surrounding healthy cortex.

Ashcroft (1), studying canker development on black walnut, reported a similar reaction of this host. He found, with the resumption of cambial activity in the spring, that the dead bark was separated from the living bark by the formation of a phellogen (cork cambium), which extended from the cambium outward until it became continuous with that at the periphery. He also pointed out that wounds made during the dormant period of the host and barely penetrating the corky layer furnished an avenue through which successful infection might occur. This same wound, however, offered no opportunity to the fungus during the growing season because the infected area was occluded by the formation of a periderm and eventually sloughed off.

The possible association of seasonal injury and prevalence of Nectria canker on northeastern hardwoods was believed to be of sufficient practical importance to justify the establishment of a small series of inoculations. The first inoculations were made in May, 1935, and the second series, in October of the same year. Injuries in the axils of branches were made by pulling the outer ends of the branches slowly down until cracking of the bark was evident. Inoculations were made by placing a perithecium, obtained from a canker in a living tree, in the crack caused by bending. The branch was then released and in most cases returned to its original position and the bark at the edges of the injury closed over the perithecium. Controls consisted of branches similarly bent but without the insertion of perithecia. The study was carried out at Black Brook Trail, Carroll, New Hampshire. The host species tested were yellow birch and sugar, red, and striped maples. The final notes were taken in May, 1937.

Analysis of the data obtained showed that for all species the 61 May inoculations resulted in only 1 definite infection as compared to 7 out of the 40 October inoculations. The 20 controls for the May and the 20 controls for the October inoculations gave no indication of Nectria infection. A comparison of the relative amount of occlusion occurring is also of interest. Seventy-five per cent of the injuries inoculated in May and 90 per cent of the May control injuries were almost completely occluded by callus. However, only 12 per cent of the injuries inoculated in October and 20 per cent of the October control injuries were similarly occluded by callus formation. Many of the October and a few of the May inoculated and control injuries remained as open wounds with good callus. In some cases the injuries caused by bending were severe and the branches died. Careful examination of the dead branches and open wounds with good callus failed, however, to show any *Nectria* fruits, and their condition was considered as resulting from injury rather than infection. Positive infections from inoculation were based on definite lesions and the presence of *Nectria* perithecia of the same species as was used in the inoculations. The results from these inoculations showed that injuries resulting from bending of branches in the fall of the year are more serious and favor Nectria infection more than similar injuries made in the spring.

DISCUSSION

Axillary Nectria cankers are common in our hardwood forests. The bark at the axils of branches is often more or less deeply fissured. It appears that cracks at the branch axils are points of entry for the canker-forming *Nectrias*. The writers' experiments show that bending of branches in the same manner as with heavy loads of ice or snow will make cracks near or at the axils, which are deep enough for *Nectria* to infect. Such injuries by ice or snow occur at the seasons when the tree is in the dormant or near-dormant condition and when canker *Nectrias* are best able to infect them. The above results suggest that reduction of canker formation will be effected by lessening the number of weak side branches in early life of the hardwoods. Practically, this can be done by favoring the growth of young reproduction at a density such that lower branches are shaded out early (2). Moreover, a large tree population gives an opportunity for selecting suitable crop trees in weeding and thinning operations.

SUMMARY

Field studies show conclusively that branches are intimately associated with Nectria-canker formation on the trunks of northeastern hardwoods. Observations also show that small young branches, buds, and short spurs, particularly on the birches, often serve as avenues of entry for the canker fungus. Branches over ½ inch in diameter do not serve as avenues of entrance for Nectrias into larger branches or trunks unless infection occurs at or very close to their axils.

Inoculation studies in conjunction with field observations strongly indicate that infection usually occurs through living or dying branches rather than through completely dead branch stubs. The size of the branch attacked, the time of year that injury occurs, and host reaction are important factors in canker development. In general, small stems are more readily girdled than large ones, and fall and winter injuries appear more important than injuries occurring in the late spring or early summer, when prompt activity of the cork cambium may help to check early stages of invasion by the canker fungus.

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VERTICILLIUM WILT OF PEPPER, CAPSICUM ANNUUM[1]

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INTRODUCTION

The occurrence of a fungus wilt of pepper, *Capsicum annuum* L., has been reported recently from California[2] and Connecticut.[3] In these instances *Verticillium* was isolated consistently, frequently in a pure state, from the vascular tissue of diseased plants obtained from the field. The Anaheim Chili and Mexican Chili varieties were affected in California, and the Worldbeater and California Wonder varieties in Connecticut. Owing to these circumstances, and to the additional fact that the symptoms were those of a vascular mycosis, it has been suggested that the *Verticillium* isolated was in fact the pathogen. However, since there is reference in the literature to unsuccessful attempts to infect pepper with *Verticillium,* and no experimental data produced in this country proving the susceptibility of this plant to attack by it, the writers deemed it desirable to test the pathogenicity of the California isolate. The results, given here, serve to demonstrate clearly the susceptibility of *C. annuum* to this pathogen.

In Europe, Bewley (1, 2, 3, 4) has reported the successful inoculation of pepper, *Capsicum* sp. with *Verticillium albo-atrum* R. and B. from tomato. Wollenweber (12) has listed *Capsicum* sp. as a host of this fungus but has given no experimental data as evidence. On the basis of Wollenweber's listing, van der Meer (11), together with Dufrénoy and Dufrénoy (7), cites the species as *C. frutescens* Willd. for reasons not given. A Verticillium disease of pepper, *C. annuum,* was described by Curzi (5, 6) who gave the name *Verticillium tracheiphilum,* n. sp. to the pathogen. He reported that the fungus was capable of attacking egg plant also.

No definite report of *Verticillium* hadromycosis of pepper has been seen in American literature other than the two referred to above.[2, 3] Haenseler (8, 9) was unable to infect either pepper or tomato with *Verticillium* isolated from eggplant. Ludbrook (10) also was unable to infect pepper in tests involving a number of isolations of *V. dahliae* Klebahn and *V. albo-atrum* from different hosts.

PATHOGENICITY

The inoculation trials were performed in the greenhouse during the winter months, when a temperature of approximately 70° F. was maintained. Seed was sown in 6-inch pots of soil that had been sterilized in an autoclave. The fungus, previously isolated from diseased peppers of a commercial planting, was grown in pure culture on potato-dextrose slants. At the time of inoculation the spores and mycelium from 2 slants were worked into the top inch of soil about the plants of each pot. The varieties Anaheim Chili and Red Chili were used. Six pots, with 3 to 5 plants each, were planted to each variety. Three of the pots of each variety were inoculated, and the others kept as controls.

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[1] The assistance of nontechnical employees of the Works Progress Administration is acknowledged.

[2] Rudolph, B. A., and W. C. Snyder. Verticillium wilt of pepper. Plant Dis. Rep. 21: 404. 1937.

[3] Dunlap, A. A. Rust on teosinte and wilt of peppers in Connecticut. Plant Dis. Rep. 21: 426. 1937

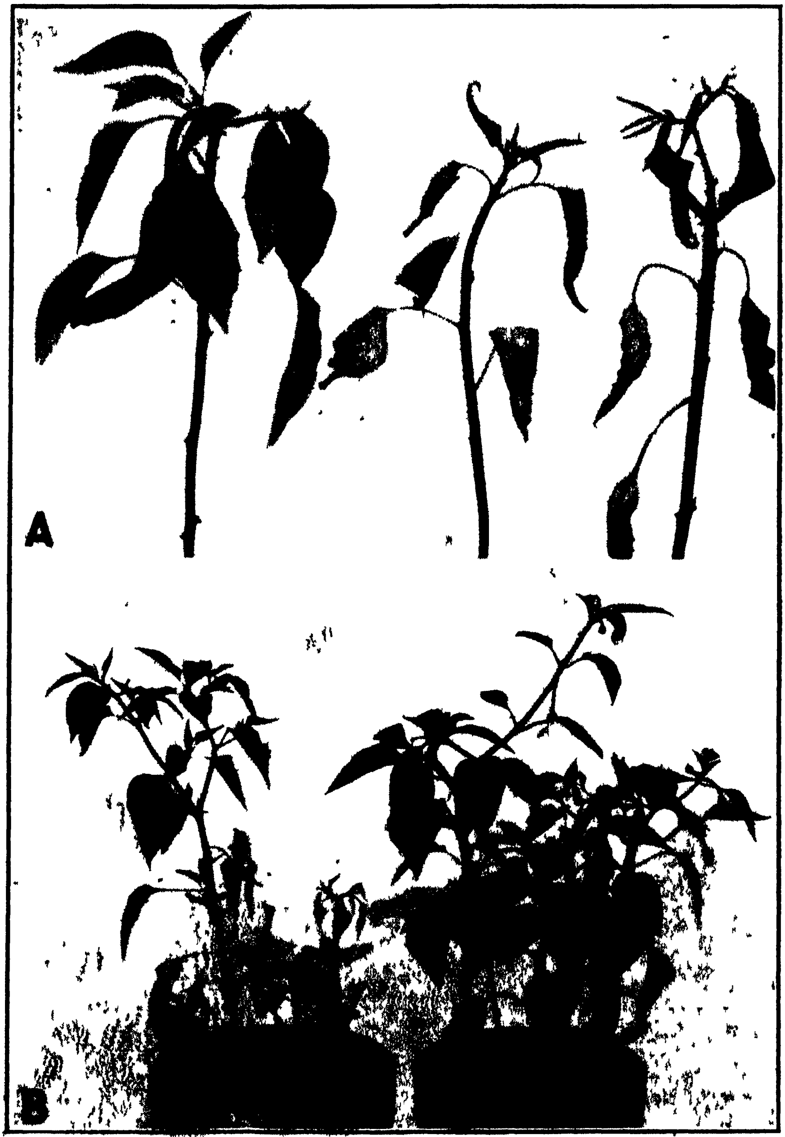

FIG. 1. Verticillium wilt of Anaheim Chili pepper. A. The plant on the extreme left is a healthy control. The fungus was recovered from the tips of the two diseased plants. B. All plants in the pot on the left are infected. The control pot is on the right.

Ten weeks after introducing the *Verticillium* into the soil, symptoms of disease were well expressed. The infected plants were stunted and the leaves were prematurely yellow and rolled inwardly. They were beginning to wither progressively inward from the leaf tip. Many of the flower buds of these plants had dropped. In contrast, none of these symptoms appeared in any of the non-inoculated pots.

At this time a slight difference in symptoms between Anaheim Chili and Red Chili was noted. The leaves of the former rolled less tightly than the latter, but showed symptoms of wilting through the entire height of the plant. The wilting or drooping of the leaves was followed by blanching, then withering. Symptoms on the Red Chili variety had appeared only on the lower leaves, and were neither so extensive nor so severe as on the Anaheim Chili variety.

The photographs in figure 1 were taken 3½ months following inoculation. At this time, all plants of the Anaheim Chili in the inoculated pots were diseased and most of these plants were nearly dead, without having produced any blooms or fruit. In several instances the top of the stem, with attendant leaves was brown and shriveled (Fig. 1, A). An occasional plant grown in infested soil approached the healthy plants in size, but even these showed distinct leaf symptoms (Fig. 1, B). The control plants showed no disease symptoms and produced many blooms and fruit. Inoculated Red Chili plants still showed milder symptoms than Anaheim Chili at this time; in fact, throughout the experiment Red Chili never developed the disease to the extent of the Anaheim Chili.

When the stems of the diseased plants were cut and viewed in cross section, a dark brown discoloration of the vascular ring appeared as a characteristic symptom. The cortex that covered this discolored vascular tissue bore no outward evidence of disease.

Tissue platings made at various intervals along the stems of diseased plants yielded only the one fungus, *Verticillium albo-atrum*. It was readily recovered from the discolored vascular cylinder at the top, as well as at the base of the plants.

DISCUSSION

The experimental data reported here, representing 10 to 15 plants each in the inoculated and control series, respectively, in both trials, is so clear-cut as to leave no doubt in the writers' minds as to the high degree of susceptibility to verticilliosis that exists in *Capsicum annuum*, especially the Anaheim Chili variety. All of the symptoms characteristic of *Verticillium* hadromycosis (12) were obtained in a relatively short period and with a high degree of severity by introducing pure cultures of the fungus into the soil about young pepper plants. It was found possible to recover the same fungus in pure form from the vascular tissue at the tips of the diseased plants.

When a planting of Dwarf Champion tomatoes was made in the pots in which the *Verticillium*-infected peppers had grown, no satisfactory aerial symptoms appeared within 4 months. However, on culturing the root systems of these plants, *Verticillium* was obtained from a number of small side rootlets. The vascular elements of the tap roots and main stems were never discolored nor was the fungus ever isolated from them.

As has been indicated elsewhere[4] (8), verticilliosis of pepper has proved to be an important disease in fields where it has appeared. Possibly *Verticillium* rather than *Fusarium* has been the cause of some of the vascular mycosis attributed to the latter fungus in the past.

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INHERITANCE OF IMMUNITY FROM VIRUS X (LATENT MOSAIC) IN THE POTATO

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(Accepted for publication January 17, 1939)

The group of potato mosaics discussed in this paper was named latent mosaic in the earlier researches because certain varieties showed no pathological reactions when infected with these viruses alone. Under some conditions infected plants of Green Mountain, Irish Cobbler, and Triumph manifest a faint mottling and a slightly rugose appearance of the leaves, but under ordinary field conditions these varieties appear healthy, that is, they are latent carriers.

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[4] See footnote 2.

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This group of viruses was later designated as virus X by Smith (7) and still later as Solanum virus 1 (8). Strains of virus X have been distinguished by their reactions on potato varieties and Jimson weed, *Datura stramonium.*

At least 4 strains can be distinguished on Jimson weed. With one strain no pathological reactions are apparent, with another only a faint mottling can be seen, the third causes a more distinct mottling, and the fourth results in necrosis. Various reactions have been observed also on potato varieties, indicating at least 6 distinct strains. Schultz *et al.* (2, 4, 5) have shown that in grafts and field exposure to infection, varieties may be grouped as immune, latent carriers, mottled, and necrotic. On the basis of rate of infection in field exposure tests, they may be grouped as immune, rarely infected, and readily infected. These various reactions may, in part, be attributable to different strains of X, but some of them are traceable to genetic differences between varieties. The X strains interact, also, with other viruses (3) resulting in such well-known diseases as mild mosaic, caused by viruses X + A, and rugose mosaic, caused by viruses X + Y. In fact, it was in tuber grafts with potatoes infected with these two mosaics, that immunity from virus X was first observed (2). It was found that one seedling, 41956, when grafted with mild mosaic, contracted only A but not X, and when grafted with rugose mosaic it became infected with Y but, again, not with X.

Resistance to virus X was determined by shoot grafting latent-mosaic Green Mountain onto plants of the seedling varieties to be tested. The formation of aerial tubers on the Green Mountain scion was considered an index of immunity (1, 6).

The grafted seedlings that did not give the aerial tuber reaction were further tested by sap inoculations on Jimson weed and pepper to determine whether or not they were latent carriers of the virus.

The inheritance of immunity from virus X, of S 41956, in graft tests has been studied in the F_1 of two crosses, S 41956 × Katahdin, and S 41956 × Earlaine, in two F_2 progenies resulting from selfing an immune seedling selection from each of the crosses, and in a progeny of S 41956 selfed.

The data for immunity and non-immunity of the various progenies and checks are given in table 1. In the F_1 progeny of S 41956 × Katahdin, 75 of the 203 seedlings, or 37 per cent, were immune, if aerial tuber formation is taken as the criterion of immunity. The same percentage of immune seedlings were found also in the F_1 of S 41956 × Earlaine. The progeny of an immune selection of S 41956 × Katahdin, when selfed, gave 94 immune seedlings, or 78 per cent of a total population of 121. An immune selection of the cross S 41956 × Earlaine selfed, gave approximately 72 per cent immune seedlings, as did also a progeny of S 41956 selfed.

That comparatively large percentages of the progenies of the crosses and selfed lines are immune is gratifying to the plant breeder, since it should not be difficult to combine this character with other characters of economic importance.

The genetic implications are interesting, also. The varieties used are evidently autotetraploids with the regular type of tetrasomic inheritance operative. The results can be explained by assuming that the genetic constitutions of the immune S 41956 and of the two immune seedlings 774–67 and 792–114 are AA aa Bb bb in which genes A and B are both necessary for immunity. Katahdin and Earlaine, on the other hand, would be genotypically aa aa bb bb.

The calculated ratios based on these assumptions fit the observed reasonably close, since the deviations divided by the standard errors are less than 2 in every case. Work is being continued to test further the validity of the hypothesis.

TABLE 1.—*Immunity and non-immunity in a number of progenies and check plants stem-grafted to virus X Green Mountains (The observed and calculated ratios are given)*

Pedigree number or variety name	Parentage	Reaction of parents	Reaction of seedlings				
			Immune		Non-immune		Total
			No.	*Per cent*	*No.*	*Per cent*	*No.*
774	S. 41956 × Katahdin	Immune × Non-immune	75	37	128	63	203[a]
774	do	do	85	42	118	58	203[b]
792	S. 41956 × Earlaine	do	50	37	85	63	135[a]
792	do	do	57	42	78	58	135[b]
774–67	Selection from 774 selfed	Immune	94	78	27	22	121[a]
774–67	do	do	88	73	33	27	121[b]
792–114	Selection from 792 selfed	do	47	72	18	28	65[a]
792–114	do	do	47	73	18	28	65[b]
	S. 41956 selfed	do	58	72	23	28	81[a]
	do	do	59	73	22	27	81[b]
Katahdin					6		6
Earlaine					4		4
S. 41956			10				10

[a] Observed numbers.

[b] Calculated on the hypothesis of tetrasomic inheritance with the genetic constitution of the immune parents AA aa Bb bb and the non-immune aa aa bb bb and assuming that both A and B are necessary for immunity. In every case the deviation of the observed from calculated divided by the standard error is less than 2.

SUMMARY

There are at least 6 strains of potato virus X, causing latent mosaic. S 41956 is immune from them all in graft tests.

The inheritance of this immunity has been studied in crosses between S 41956 and two non-immune varieties; in a progeny of S 41956 selfed; and in progenies of two other immunes selfed. Thirty-seven per cent of the progenies of the two crosses and from 72 to 78 per cent of the selfed lines, were found to be immune. The results can be explained by the usual type of inheritance of autotetraploids.

It is assumed that with the genes A and B both necessary for immunity the immune plants used as parents in this study have the genetic constitution AA aa Bb bb, the non-immune aa aa bb bb.

The ratios calculated on the basis of this hypothesis fit the observed reasonably close in all the progenies. In no case does the deviation, divided by the standard error, exceed 2.

Work is being continued to test further the validity of the hypothesis.

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ERGOT OF PASPALUM

C. L. LEFEBVRE

(Accepted for publication December 5, 1938)

As a result of observations and artificial inoculations made during the past 2 years, several species of *Paspalum* not previously reported as hosts of the ergot fungus, *Claviceps paspali* Stevens and Hall, have been found susceptible to this parasite. In the summer of 1936, Dr. G. W. Burton found a single head of *P. urvillei* Steud. infected with ergot in the grass nursery of the Division of Forage Crops and Diseases at Tifton, Georgia. Again, in 1937 and 1938 several infected heads of the same species were found in this nursery. Ergot infection was observed by Dr. Burton also on *P. notatum* Flügge at Tifton, Ga., in 1938, for the first time during the 3 years he has had this species under observation.

In the spring of 1938, a nursery of 23 species and strains of *Paspalum* was established at Arlington Experiment Farm, Arlington, Virginia, by transplanting seedling plants from the greenhouse. As each species or strain reached the flowering stage, 100 heads were inoculated artificially with a conidial suspension of the ergot fungus obtained from naturally infected *P. laeve* Michx. at Arlington, Va. As a result of these inoculations made during the month of July an epidemic of ergot developed in the nursery apparently through insect dissemination of the inoculum. Observations were made at intervals during the summer on the relative suceptibility and resistance of the species and strains of *Paspalum* present.

In this nursery approximately 90 per cent of the late-formed heads of *Paspalum urvillei* were found to be infected with ergot. There were 3 strains of this grass, each occupying a row 100 feet in length, the plants having originated from seed received from Tifton, Ga.; Gainesville, Florida; and Fayetteville, Arkansas, respectively. The 3 strains seemed to be equally susceptible. While a high percentage of the heads were infected, as indicated by the presence of abundant dried up "honey dew," only comparatively few florets were found bearing sclerotia. The sclerotia in the florets of *P. urvillei* were spherical to ellipsoid and measured 1–2 mm. in length by 1–1.5 mm. in diameter, while those found in the florets of *P. dilatatum* Poir. and *P. laeve*, usually measured 3–4.5 mm. in diameter. Also, the smaller sclerotia on the former grass were not so irregular and deeply ridged as those on the latter grasses. Although the ergot epidemic started in July, *P. urvillei* remained ergot-free until late September, even though the more susceptible species near by were heavily infected throughout the summer.

Paspalum longipilum Nash also was heavily infected with ergot in this grass nursery. Previous observations, however, showed it to be free of ergot when growing in a mixed stand with heavily infected *P. laeve* in an open field on the Arlington Experiment Farm. *Paspalum pubescens* Muhl. was found growing in this same field but it also was free of ergot, although it was readily infected when heads were inoculated in the greenhouse with apparently the same strain of ergot that was so prevalent in the field.

Ergot infection was found only on the late formed heads of *Paspalum langei* (Fourn.) Nash, and *P. ciliatifolium* Michx., even though the ergot organism was abundant in adjacent rows of other species heavily infected throughout the summer. *P. ciliatifolium* seemed to be resistant, at least to this particular collection of ergot, as only 16 infected heads could be found in a 100-ft. row. Observations made at the same time showed that *P. langei* was even more resistant than the above species, as only 10 infected heads were found in a row of the same length. Furthermore, only 1 or 2 sclerotia usually were found in each infected raceme of *P. langei*, while as many as 14 sclerotia were found in 1 infected raceme of *P. ciliatifolium.*

In 2 rows of *P. plicatulum* Michx., each 100 ft. in length, 50 heads were inoculated in each row, but only 5 heads were observed producing "honey dew," and in no case were sclerotia formed. This species apparently is extremely resistant to this collection of ergot.

Similary, *Paspalum notatum* was inoculated in the field and in the greenhouse but no infection was secured. Therefore, this species apparently is highly resistant to the collection of ergot used in these inoculations. It is not immune from ergot, however, as is evident by the few infected heads found at Tifton, Ga., and from the several small specimens found in the mycological collections of the Division of Mycology and Disease Survey, Bureau of Plant Industry.

In the greenhouse, infection was readily secured on several grasses not included in the nursery, namely, *Paspalum distichum* L., *P. pubiflorum* Rupr., *P. floridanum* Michx., and *P. intermedium* Munro.

The following species were repeatedly inoculated in the field, but no infection resulted: *P. notatum* Flügge (narrow leaf-type from Paraguay), *P. notatum* Flügge (common local type from Georgia), *P. lividum* Trin., *P. malacophyllum* Trin. (a strain from Tifton, Ga. and one from Gainesville, Fla.), and *P. supinum* Bosc.

So far as the writer is aware the following are new hosts of *Claviceps paspali: Paspalum urvillei* Stud., *P. longipilum* Nash, *P. pubescens* Muhl., *P. pubiflorum* Rupr., *P. ciliatifolium* Michx., *P. langei* (Fourn.) Nash, and *P. intermedium* Munro.

Specimens of ergot on these species of *Paspalum* have been deposited in the mycological collections of the Division of Mycology and Disease Survey, Bureau of Plant Industry.

DIVISION OF FORAGE CROPS AND DISEASES,
BUREAU OF PLANT INDUSTRY.

PHYTOPATHOLOGICAL NOTES

Damping-off as a Factor in the Natural Distribution of Pine Species.—The distribution of pine species in Wisconsin shows a striking correlation with soil conditions. The southern portion of the State, roughly delineated by Hudson, Baraboo and Manitowoc, is predominantly covered with heavy loam soils derived from calcareous drift of recent glaciation or from residual limestone. Throughout this area red pine does not occur naturally. White pine is distributed sporadically on shallow ridge soils underlain by sandstone or quartzite. Jack pine is confined to isolated localities on coarse sandy soils of the Wisconsin River terraces. At the same time, all three of these species form extensive stands north of the line of calcareous region, particularly on the vast plain of the previous "Glacial Lake Wisconsin."

An excess of calcium and magnesium carbonates, the unavailability of iron, and absence of certain rare elements are the soil factors which are often thought to be responsible for the absence of pine species on the calcareous deposits of Southern Wisconsin. However, the thrifty growth of some stands of red and white pine, artificially planted in this region, greatly depreciates the validity of theories assuming nutrient deficiency or toxicity of soils. This suggests that among the factors limiting the natural southward distribution of pines, a prominent rôle is played by the destructive activity of damping-off fungi, prevalent on heavy mull soils of calcareous origin. Further evidence in favor of this concept was obtained in an ecological study of the University of Wisconsin Arboretum, near Madison.

Samples of representative soils from the Arboretum area were collected for a greenhouse study of their fertility and adaptation to various tree species. The study was carried on in half-gallon porcelain jars, in series of

4. Twenty-five seeds were sown per pot. A record of germination and damping-off was kept for each species seeded, and significant results were obtained with respect to red pine (*Pinus resinosa*), white pine (*P. strobus*), and Austrian pine (*P. nigra*).

On sandy soils, occurring in the arboretum in small eroded patches, none of the pine species suffered losses from damping off greater than 8 per cent.

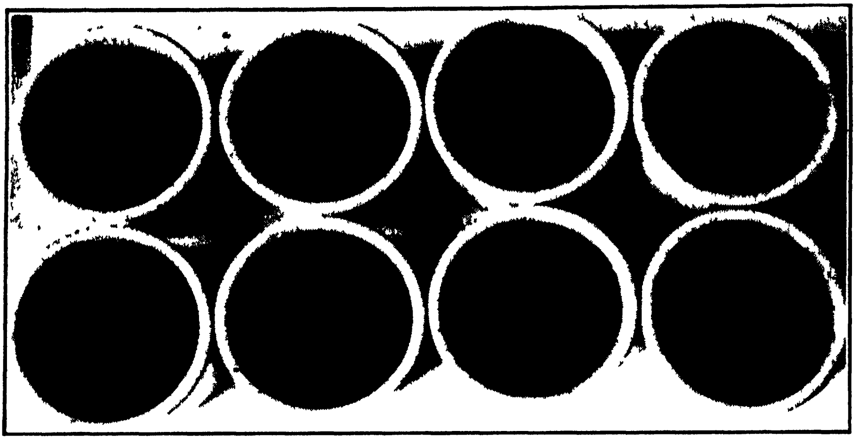

FIG. 1. Effect of soil upon damping-off of red pine, *Pinus resinosa*. Jars on the left contain siliceous soil of sandy texture whereas jars on the right, mull loam derived from calcareous drift; damping-off losses 4 and 96 per cent, respectively.

On heavy mull soils red pine was almost completely destroyed, the pre-emergence and post-emergence losses amounting to 96 per cent (Fig. 1). White pine suffered on the same soil to a considerably lesser degree, while Austrian pine was not affected at all.

The soils in question are characterized by the analytical data in table 1.

TABLE 1.—*Composition of the upper 7-inch layer of the eroded sandy loam and nut-structured mull loam soils from the University Arboretum near Madison, Wisconsin*

Constituents / Soil types	Reaction pH	Clay fraction per cent	Base exchange capacity ME/100 g.	Total N per cent	Avail. P_2O_5	Avail. K_2O	Repl. Ca	Repl. Mg
					p.p.m.		ME/100 g.	
Eroded sandy loam	5.8	7.4	6.4	.056	40.5	92.00	3.70	1.60
Nut-structured mull loam	6.3	17.3	20.4	.228	73.7	172.27	12.67	2.93

The results obtained coincide closely with the present empirical knowledge of the relative resistance of pines to damping-off fungi. Red pine, as well as jack pine, is known to be extremely susceptible to infection, while white pine is considered quite resistant to this disease. Austrian pine often suffers great losses on acid soils of forest nurseries. However, it is likely to be less susceptible to infection on heavy soils derived from calcareous rocks, since such soils are the native habitat of this species.

In the light of these findings, no matter how incidental they are, it is appropriate to question the advisability of extensive plantings of northern conifers on heavy soils of calcareous origin. Such species may never be able to regenerate naturally and, hence, will be doomed to extinction or costly clear-cut management. The principal argument that may be advanced against this assumption is the possibility of the future acidification of the soil by coniferous leaf litter and a gradual reduction in the virulence of damping-off organisms. However, such a modification of soil conditions would take place only provided the trees be planted at the close spacing of 4 by 4 feet, eliminating the growth of hardwood species. This type of planting program would be contrary to all modern silvicultural trends toward the development of mixed hardwood-coniferous stands.—S. A. WILDE AND D. P. WHITE, *Department of Soils, University of Wisconsin.*

Currant Mosaic.—This is a preliminary statement on what appears to be a new virus disease on currant.

In the spring of 1935 the writer's attention was attracted to a striking chlorotic condition on currants (*Ribes rubrum* L.) in a commercial planting in Ulster County, New York. Inquiry revealed that this trouble had been present in the county for several years, but that it had aroused no special concern owing to its limited occurrence and its slow rate of spread. So far as we are aware this condition is limited to the Hudson River Valley, although a similar if not identical trouble was observed by H. H. Whetzel on currants in his garden at Ithaca several years back. These plants became unproductive and were removed. No other cases have since been noted.

Currant mosaic has recently assumed importance by virtue of its rapid spread in the above commercial planting in the past 4 seasons. Affected plants were located in but a few rows on one side of the field of between 3 and 4 acres in 1935. By 1938 it had spread completely across the planting of over 100 rows, affecting a high percentage of the plants.

The most striking symptom is the chlorotic patterns on the leaves (Fig. 1). In its earlier stages light green, irregularly circular spots appear, principally along the midrib and the larger veins. These spots enlarge and join, producing a gradual clearing of the leaves along the veins. The vein banding, if such it may be called, is extremely irregular.

The disease appears not to spread in the plant after the leaves reach maturity. The extent of chlorosis on individual leaves, as well as on individual shoots and plants, is variable. One side of the plant may be severely affected, while the other may have only occasional affected leaves. It is unusual for entire leaves to become chlorotic. Their affected light green areas gradually change to white, a stage prevalent in late June and early July. Later in the season the clear areas and particularly those at the leaf margins may die,

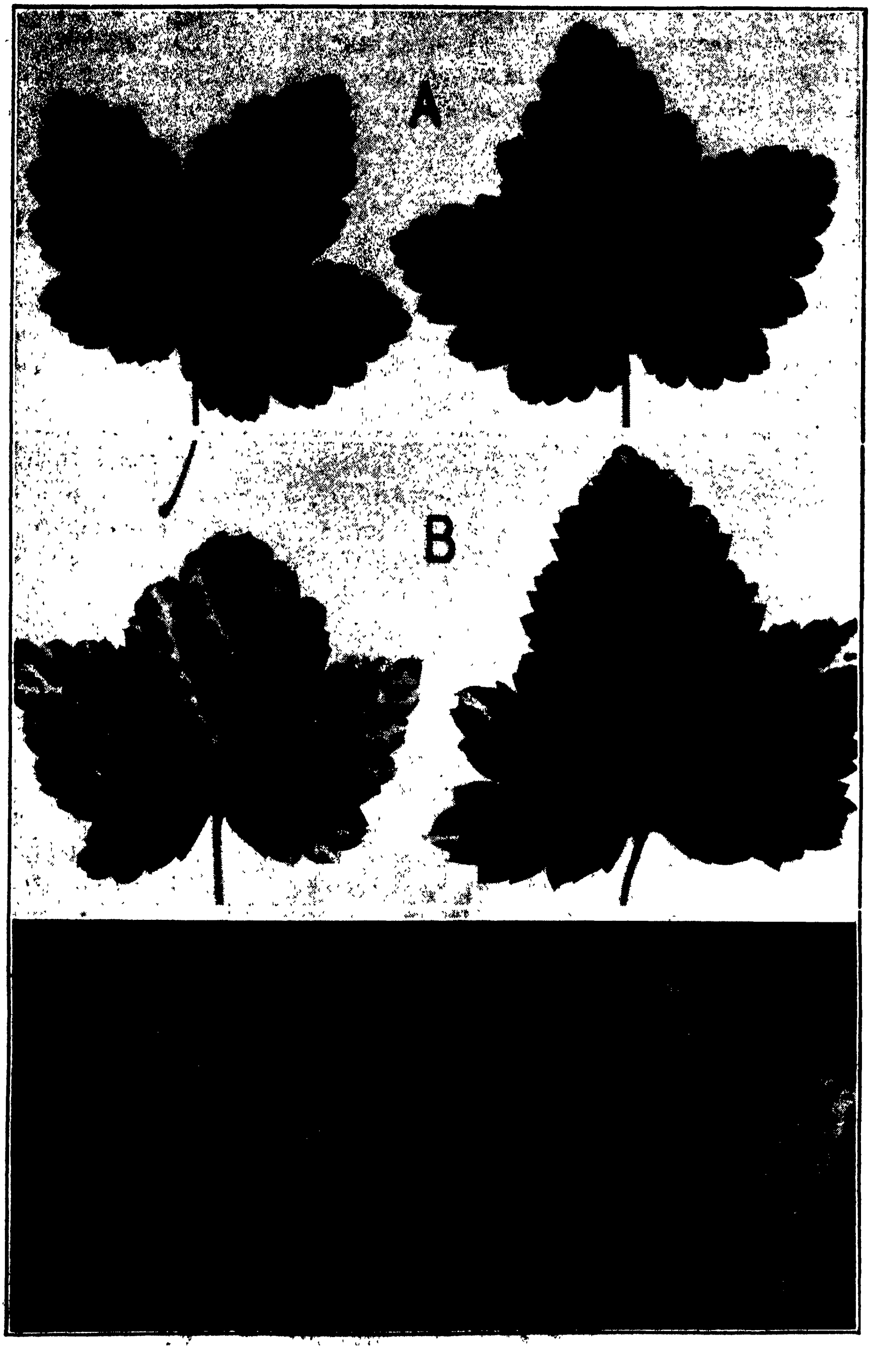

FIG. 1. Symptoms of mosaic on red currant. A. Early stage. Light green irregularly circular spots appear along the veins. These spots have a tendency to enlarge and run together. B. Later stage. In late June the leaf symptoms vary from slight to severe clearing along the veins, which results in irregular patterns that somewhat resemble vein banding.

turn brown, and give the appearance of chemical injury. The first evidence of such disintegration usually begins to appear in July. The more severely affected leaves may fall by mid-August, causing defoliation in varying degrees. The presence in this planting of 2 rather prevalent leaf-spot fungi (*Mycosphaerella grosulariae* and *Pseudopeziza ribis*), which also cause defoliation, has complicated the problem of accurately evaluating this point.

Stunting marks the more advanced stages of the disease, and affected plants gradually decline in vigor and fruitfulness and may die after several years. In their decline dieback may be local or general. The one-sided appearance, sometimes encountered, results from the fact that the more severely affected branches were localized on one side of the plant. There are no evidences of recovery from the disease but early pruning of diseased shoots seems to check its spread.

Transmission experiments by budding and whip grafting have failed to produce infection on healthy plants. A limited number of trials were conducted in 1935, 1936, and 1937, all with negative results. The reason for failure is not clear but must be associated in some way with the rather general failure in obtaining good unions between stock and scion. This hypothesis was tested experimentally.

When good unions were secured by inarching together vigorously growing shoots of healthy and diseased plants, successful transmission resulted. The grafts were made in the greenhouse in July, 1937. About 2 months later, in September, the plants were placed outdoors for hardening. Excellent unions were then noted; and for each graft the connection to the diseased plant was severed below the union. When the plants were returned to the greenhouse in mid-January it was observed that the exposure had killed the scions; otherwise, the plants were all right. Although but 5 such grafts were made, transmission resulted in 3 cases, a typical specimen of which is shown in figure 1A.

Attempts to propagate from cuttings of diseased plants failed, whereas fair success was achieved with healthy cuttings; another indication of how the vitality of the plant is lowered by the disease.

While the natural dissemination of currant mosaic is assumed to be by an insect, transmission experiments have not yet succeeded in determining its identity.—E. M. HILDEBRAND, *Department of Plant Pathology, Cornell University, Ithaca, New York.*

"Cephalosporium Die-back" of Elms.—"Cephalosporium die-back" of elms, recently determined by Verral and May[1] as attributable to *Dothiorella ulmi,* is the disease most frequently found in the specimens of elms received at the Connecticut Agricultural Experiment Station. This fungus is known to be very virulent, but 2 elms in New Haven, Conn., have been infected with this organism for 12 growing seasons (11 years), and are, therefore, of interest.

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[1] Verrall, A. F., and C. May. A new species of *Dothiorella* causing die-back of elm. Mycologia 29: 321–324. 1937.

On August 5, 1927, branches from these trees were brought to this laboratory. Some of the leaves were withered, and there were definite discolored streaks in the wood. Sections made by the writer showed a fungus in the streaks and repeated cultures made that year and later produced the same fungus. Other work prevented the writer from inoculating healthy elms with this fungus until Jan. 30, 1930, when the first of more than 125 young elms in the greenhouse were inoculated. These inoculations were made in different ways, but chiefly by putting some of the fungus into cuts made on the stems and covering the wound with moist sterilized cotton. In some cases the mycelium was planted in the soil at the base of the tree. The leaves of 1 inoculated tree withered in a way resembling the withered leaves on the trees from which cultures were obtained; but, after a time, this plant recovered and healthy leaves appeared. In other cases discolorations about 1 inch long appeared in the wood, and in 4 cultures the fungus was reisolated from such streaks. On the whole, the response to the inoculation was so slight that there was published in 1931 only a short notice.[2]

Curtis May published in 1931[3] an account of the same fungus, which he had isolated from trees in widely separated localities. Seven out of his 14 inoculations on young elms were successful. The symptoms were similar to those of the diseased trees and there followed the death of the branches above the point of inoculation. A culture of the fungus isolated from the New Haven trees was submitted to Dr. May who pronounced it to be the same organism as the one he had isolated.

The 2 New Haven trees seem worth noting, since during the 11-year period 16 cultures made at different times and from different branches have yielded the same fungus. The latest culture was made July 12, 1938. The trees were then in as good, if not better, condition than when the first cultures were made, in 1927. They also have withstood the destruction of the hurricane. In the beginning of each summer there has been the normal appearance of foliage, but, about the middle of June, scattered branches showed withered leaves. Later in the season, after such withered leaves have fallen, the trees again assume a relatively healthy appearance.

Two possible explanations of the survival of these trees may be considered. The trees may have some resistance to the fungus or the fungus strain may be only mildly parasitic. The second deduction seems more likely, since the fungus has been shown elsewhere to be highly virulent and also, since inoculations of the fungus isolated from these trees produced only slight infections. Whatever the factor may be it seems well worth while to put on record 2 trees that are known by cultures to be infected with the same fungus for so long a period.—FLORENCE A. MCCORMICK, Connecticut Agricultural Experiment Station, New Haven, Conn.

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[2] Slate, W. L. Elm disease under investigation. Conn. Agr. Exp. Sta. Bull. 322 (1930): 119. 1931.

[3] May, C. A new elm disease. Science (n. s.) 74: 437. 1931.

Forced Ejection of Ascospores from Apothecia of Sclerotinia Species.—While preserving apothecia of *Sclerotinia sclerotiorum* (Lib.) Mass., it was noticed that exposure of mature apothecia to the vapors over alcohol-formalin-acetic acid No. 2[1] induced repeated ascospore discharge. The same effect was observed when brown-rot apothecia were similarly exposed. This solution consists of approximately the following percentages by volume: Alcohol 41.7, formalin 14.3, acetic acid 14.3, and water 41.7. Separate solutions of alcohol, formalin, and acetic acid of these concentrations were made up in distilled water. Mature apothecia, which had been stored in a moist chamber at 4° C. for two days, were exposed successively over each of these solutions and over the complete fixing solution.

Vapors of alcohol and acetic acid had little or no effect on ascospore discharge, but exposure for 1 to 4 seconds over either the formalin solution or the complete fixing solution induced violent and often repeated ejection of ascospores. Spores thus released were caught on inverted agar plates and in distilled water. In either case a large percentage of the captured spores germinated, indicating that the vapors were not lethal to the ascospores.

The above method has been found superior to the usual methods for inducing ascospore discharge, and it is suggested for trial when an abundance of ascospores are wanted for observation or inoculation, or when it is desired to exhibit ascospore discharge.—L. D. LEACH AND WM. B. HEWITT, *University of California, College of Agriculture, Davis, California.*

The Occurrence of the Perfect Stage of Rhizoctonia solani in Platings of Diseased Cotton Seedlings.—During the course of a survey of the fungi associated with seedling diseases of cotton a sample of several young plants was obtained that exhibited rather unusual symptoms. These plants, while they did show typical lesions about the base of the hypocotyl, were characterized by a wilted and water-soaked condition of the youngest leaves and petioles.

The plants were thoroughly washed, surface-disinfected in a solution of sodium hypochlorite, and plated out on tap-water agar. After 2 days of incubation at 27° to 29° C. the mycelium of *Rhizoctonia solani* Kühn was observed growing from the pieces of plated tissue. Four days after plating grayish-white, pulverulent areas were found on and immediately adjacent to the seedling pieces. When observed under the microscope numerous small oblong spores were seen on the surface of the agar. Further focusing revealed a considerable number of basidia bearing typically 4, but occasionally 2, basidiospores. (Fig. 1.) The size, shape, and general appearance of the basidia, sterigmata, and basidiospores conformed to those described for *Corticium vagum* Berk. and Curt.

Preliminary attempts were made to reproduce the complete life cycle of the fungus with transfers from this isolate. Mycelial, monospore, and multispore transfers were made to plates of tap-water agar containing a few pieces of healthy cotton seedlings that had been surface sterilized. Cultures arising from the mycelial transfers, as well as those derived from single basidiospores produced abundant mycelial growth, but failed to give rise to the basidiomycetous stage. In those cultures derived from mass transfers of basidiospores, however, basidia and basidiospores were observed after 4 to 6 days on and around the seedling pieces lying on the surface of the agar.

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[1] Rawlins, T. E. Phytopathological and botanical research methods. 156 pp. J. Wiley & Sons, Inc., New York; Chapman & Hall, Ltd., London. 1933.

Apparently, the isolate derived from the diseased cotton seedlings was unusually virulent. Not only were the seedlings well beyond the stage when they are ordinarily attacked by this fungus, but the symptoms produced were not typical of these generally attributed to *Rhizoctonia solani.* The particular isolate is of further interest in that the *Corticium* stage was produced under artificial conditions on a non-living substrate, and that the complete life cycle was repeated only when multispore transfers were used to seed plates of tap-water agar.—ARNOLD J. ULLSTRUP,[1] Bureau of Plant Industry, United States Department of Agriculture in cooperation with the South Carolina Agricultural Experiment Station, Clemson, South Carolina.

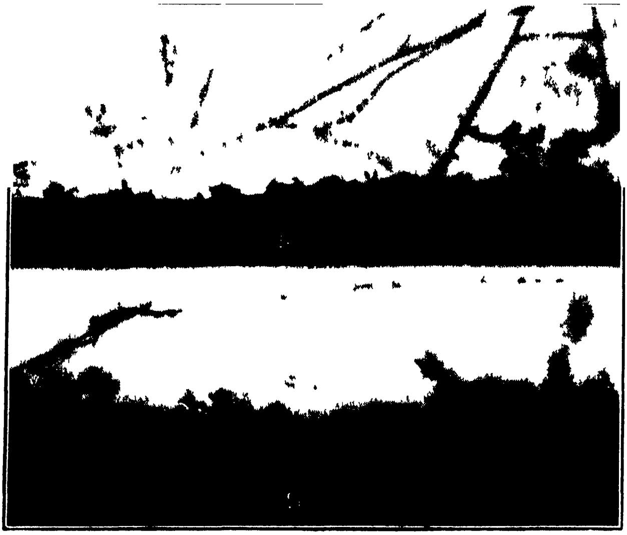

FIG. 1. Basidia and basidiospores along the edges of plated cotton seedlings (S).

BOOK REVIEW

MÜNDKUR, B. B. *Fungi of India,* Supplement 1. 54 pp. Scientific Monograph No. 12, The Imperial Council of Agricultural Research. New Delhi, 1938. 2s. 3d.

This publication constitutes the first supplement to Butler and Bisby's Fungi of India, published in 1931 as Monograph No. 1 of this series. This latter work listed 2,351 species that had been reported as occurring in India up to the year 1930. The present list adds 433 fungus species, together with 90 Myxomycetes, a group not included by Butler and Bisby, for a total of 2,874 species. One new species, *Mycosphaerella tinosporae* Ajrekar is described, *Peronospora gaumanni* is proposed as a new name for *P. indica* Gäumann, the latter specific name being preoccupied, and four new combinations are made. Recent studies made it possible to include coprophilous and soil fungi and water molds not previously recorded. 134 references to the literature bearing on Indian fungi supplemental to those of Butler and Bisby's work are appended. A host index is in preparation.—JOHN A. STEVENSON, Bureau of Plant Industry, Washington, D. C.

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[1] Formerly Agent in the Division of Cotton and Other Fiber Crops and Diseases.

REPORT OF THE THIRTIETH ANNUAL MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

THE 1938 RICHMOND MEETING

The thirtieth annual meeting of The American Phytopathological Society, held in Richmond, Virginia (December 27 to 30, 1938), was very successful and well attended. About 300 members registered. Seventy-nine new members were elected at the meeting, and this brought the active membership roll to 1077.

More than 350 attended the Phytopathologists' Dinner at the Jefferson Hotel, and enjoyed the program presented under the direction of the committee headed by Dr. F. V. Rand.

Special conferences were held on regulatory work and foreign plant diseases, plant-virus nomenclature, and extension work. The cotton pathologists held a joint session with the plant physiologists, at which time the relationship of plant physiology and diseases was discussed. The value of cooperation between different branches of plant science was brought out in joint sessions with the Potato Association of America, American Association of Economic Entomologists, Section G, A. A. A. S., and affiliated Botanical Societies, Floriculture Section of the American Society for Horticultural Science, and the Physiological Section of the Botanical Society of America and the American Society of Plant Physiologists.

The summer meeting will be held in Milwaukee, Wisconsin, June 19–24, 1939.

OFFICERS, REPRESENTATIVES, AND COMMITTEES FOR 1939

Officers:

C. R. ORTON, President (1 yr.), University of West Virginia, Morgantown, W. Va.
CHARLES CHUPP, Vice-President (1 yr.), Cornell University, Ithaca, New York.
R. S. KIRBY, Secretary (3 yrs.; term expires 1940), Pennsylvania State College, State College, Pa.
H. A. EDSON, Treasurer of the Society and Business Manager of PHYTOPATHOLOGY (3 yrs.; term expires 1940), U. S. Department of Agriculture, Washington, D. C.
H. B. HUMPHREY, Editor in Chief of PHYTOPATHOLOGY (3 yrs.; term expires 1940), U. S. Department of Agriculture, Washington, D. C.

Councilors:

J. J. CHRISTENSEN (term expires 1939), University of Minnesota, St. Paul, Minn.
E. B. LAMBERT (term expires 1939), U. S. Department of Agriculture, Washington, D. C.
H. W. ANDERSON (term expires 1940), University of Illinois, Urbana, Ill.
EUBANKS CARSNER (1 yr.; for the Pacific Div.), P. O. Box 752, Riverside, Calif.
A. N. BROOKS (1 yr.; for the Southern Div.), Box 522, Lakeland, Florida.

Representatives:

A. A. A. S. Council (1 yr.), S. A. Wingard, L. M. Massey.
Elector Group V, Division of Biology and Agriculture, National Research Council (terms expire 1940), E. C. Stakman (H. P. Barss, alternate).
Tropical Research Foundation (5 yrs.), L. R. Jones (term expires 1940).
International Union of Biological Sciences, A. G. Newhall.
Board of Editors, American Journal of Botany, G. W. Keitt (3 yrs.; term expires 1940).
Union of American Biological Societies (and Biological Abstracts), H. B. Humphrey and R. S. Kirby (*ex officiis*), G. W. Keitt, H. A. Edson, C. W. Bennett, H. P. Barss.
Members of The Council of The Third International Congress for Microbiology, B. O. Dodge, Wm. H. Martin.

Standing Committees:

Regulatory Work and Foreign Plant Diseases. C. R. Orton, Chm., H. T. Güssow, J. S. Boyce, W. A. McCubbin, R. D. Rands, J. F. Adams, E. L. Chambers, M. T. Munn.
Extension Work and Relations. Luther Shaw, Chm., Chas. Chupp, R. J. Haskell, S. L. Pierstorff, R. S. Kirby, E. C. Stakman, G. W. Keitt, W. B. Tisdale, I. L. Conners.
Coordination in Cereal and Vegetable Seed Treatment Research. M. B. Moore,

Chm., W. E. Brentzel, F. J. Greaney, J. G. Horsfall, H. A. Rodenhiser.

Standardization of Fungicidal Tests. S. E. A. McCallan, Chm., Wm. H. Martin, J. W. Roberts, K. J. Kadow, H. C. Young, J. G. Horsfall, C. E. Yarwood.

Advisory on Society Activities and Programs. J. C. Walker, Chm., R. K. Voorhees, M. W. Gardner, F. L. Drayton, A. A. Dunlap, W. J. Zaumeyer, E. L. Nixon.

Phytopathological Classics. H. H. Whetzel, Manager, H. B. Humphrey, Editor.

Necrology. A. G. Johnson, Chm., M. B. Waite.

Investments. H. A. Edson, Chm., N. E. Stevens, Chas. Brooks, Marvin E. Fowler, J. W. Roberts.

Donations and Legacies. E. C. Stakman, Chm., N. E. Stevens, J. G. Brown, N. J. Giddings, R. P. White.

New Memberships and Subscriptions. A. J. Riker, Chm., Kenneth Kadow, R. M. Lindgren, B. A. Rudolph, R. S. Kirby (*ex officio*), J. C. Carter.

TEMPORARY COMMITTEES

Auditing, Freeman Weiss, H. A. Rodenhiser.

Elections, J. W. Heuberger, J. G. Horsfall, O. D. Burke, A. A. Nikitin.

Resolutions, A. G. Johnson, W. G. Newhall, T. F. Manns.

REPORTS OF OFFICERS, REPRESENTATIVES AND COMMITTEES FOR 1938

Report of the Secretary. The Society year 1938 opened with 1053 members and closed with 1077, a gain of 24. At the Richmond meeting 79 new members were elected. Thirteen former members were restored to the active roll during the year. The Society lost 68 members, 11 by resignation, 2 by death, and 55 by suspension for non payment of dues. Of the full membership 143 are paid up life members and 19 are paying $10.00 per year toward life membership.

R. S. KIRBY

Report of the Treasurer. Statement of accounts for the year ending November 30, 1938.

Receipts:

Balance from 1937				$2563.81
Annual dues:				
1935	$ 10.00	($ 10.00 life)		
1936	5.00			
1937	26.99			
1938	2846.45	(239.34 life)		
1939	1866.13	(180.54 life)		
1940	5.50			
1941	5.00			
1942	5.00		$4770.07	
Voluntary dues			10.00	
Items for other accounts included in checks for dues:				
Sales	2.40			
Classics	2.00		4.40	
Balance from A. P. S. Dinner December, 1937			3.25	
Contributions from members for expenses of special committee, Biological Abstracts			10.00	
Reimbursement for stamped envelopes			49.32	
To replace checks returned by bank			15.00	
Total receipts				4862.04
				$7425.85

Expenditures:

Member subscriptions transferred to PHYTOPATHOLOGY:		
1936	4.00	
1937	21.99	
1938	2000.00	$2025.99
Transferred to Sinking Fund (Building and Loan)		184.76
Publication of Society material in PHYTOPATHOLOGY (June, 1937–June, 1938)		317.33

Secretarial work for Treasurer and Secretary	266.70	
Printing	151.12	
Stamps and envelopes	78.39	
Supplies	2.35	
Transferred to PHYTOPATHOLOGY for sales	2.40	
Transferred to Classics	2.00	
Contribution to Biological Abstracts for aid in circularizing letters	17.91	
Contribution to Union of American Biological Societies	25.00	
Card files for Secretary & Chmn. Membership Committee	6.17	
Copies of annual report for Council	3.95	
Part expenses of President for summer meeting	25.00	
Refund dues	2.50	
Telegram	0.38	
Collection charges on checks	2.93	
Checks returned by bank	14.75	
Total expenditures		$3129.63
Balance on hand[1]		4296.22
		$7425.85

Sinking Fund. The Sinking Fund, the income from which is used for the support of PHYTOPATHOLOGY, is obtained by deducting $5.00 from each $10.00 life-membership installment. This fund totaled $9,281.66 at the close of 1937. During the year ending November 30, 1938, it has increased to $9,466.42 and is invested as follows:

First mortgage notes deposited with the McLachlen Banking Corporation for collection ($1000.00 at 6%, 1000.00 at 5½%, $500.00 at 5%)	$2500.00
Invested with the following building and loan associations:	
Arlington & Fairfax Bldg. and Loan, 5%	1000.00
Columbia Permanent Bldg. Ass'n, 4%	500.00
District Bldg. and Loan Ass'n, 4%	1500.00
National Permanent Bldg. Ass'n, 5%	1000.00
Northwestern Savings and Loan Ass'n, 4½%	1000.00
Perpetual Bldg. Ass'n, 4½%	1000.00
Prudential Bldg. Ass'n, 4%	966.42
	$9466.42

The Lyman Memorial Fund for the Permanent Endowment of PHYTOPATHOLOGY, obtained from voluntary contributions, totaled $2709.81 on November 30, 1937. During the year ending November 30, 1938, this fund has increased to $2809.31, all of which is invested with the Brookland Building and Loan Association at 4%.

H. A. EDSON

Report of the Business Manager of Phytopathology. At the end of 1937 there were 615 subscriptions to PHYTOPATHOLOGY, including 6 complimentary. During 1938 there were 36 cancellations, 21 suspensions for non-payment and one discontinued complimentary subscription, a loss of 58. But, with 81 new paid subscriptions, there is a net gain of 23, increasing the list at the close of 1938 to 638. Of these, 189 are domestic and 449, foreign. Japan leads with 71, U.S.S.R. has 67 (an increase of 13 over last year), England remains the same at 37, Canada has 27, Germany 24, and India 21. Sixty-seven other countries or geographical units receive from one to 19 copies each. It is interesting to note that subscriptions to China dropped from 23 during 1937 to 12 during 1938.

Statement of accounts for the year ending November 30, 1938.

Receipts:

Balance from 1937		$ 3,444.59
Subscriptions:		
1936	$ 0.50	
1937	60.60	
1938	3,307.48	

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[1] Of this amount $2500.00 is deposited in a savings account at 2%. Of the total amount on hand approximately $3200.00 is due other accounts, primarily PHYTOPATHOLOGY for member subscriptions.

1939	459.12		
1940	5.50	$3,833.20	
Member subscriptions, 1936, 1937		25.99	
Member subscriptions 1938 (part)		2,000.00	
Sales of back numbers		404.05	
Advertising:			
1937	139.69		
1938	759.37	899.06	
Interest on Sinking Fund:			
First mortgage notes	141.33		
Building and loan	299.55	440.88	
Interest on Lyman Fund		165.03	
Grant from Rockefeller Institute		600.00	
From A. P. S. for publication of Society material		317.33	
Allowance on reprints by printer		357.81	
Payment for publication of Linford article		64.48	
Reimbursement for remaking engravings		5.77	
Allowance on stencil list		1.00	
Check redeposited		18.00	
First mortgage paid in full		1,000.00	
Total receipts			$10,132.60
			$13,577.19

Expenditures:

Printing, distributing and storing PHYTOPATHOLOGY:				
Vol. XXVII, No. 12 (containing Index)	$ 621.79			
Vol. XXVIII, No. 1	689.39			
No. 2	601.39			
No. 3	628.30			
No. 4	630.84			
No. 5	593.48			
No. 6	580.77			
No. 7	662.96			
No. 8	583.57			
No. 9	577.62			
No. 10	705.53			
No. 11	734.50	$7,610.14		
Postage		550.44		
Storage, 1937		48.00	$8,208.58	
Secretarial work and office expenses, Editor in Chief			290.98	
Secretarial work for Business Manager			205.51	
Secretarial work and office expenses, Advertising Manager			67.21	
Commission, Advertising Manager			118.15	
Stamps and envelopes			89.84	
Supplies			3.50	
Printing			24.51	
Inventory on back volumes			6.20	
Classification, stencil list			31.32	
Refund subscription			2.93	
Check returned for personal signature			18.00	
Collection charges on checks			0.30	
Reinvestment, Sinking Fund			1,000.00	
Total expenditures				$10,067.03
Balance on hand[2]				3,510.16
				$13,577.19

H. A. EDSON

Report of the Auditing Committee for the Year Ending November 30, 1938. The books of the Treasurer of the Society and the Business Manager of PHYTOPATHOLOGY have been examined, together with the records of the investments of the Sinking Fund and the Lyman Memorial Fund. The accounts were found correct and all records in proper order.

FREEMAN WEISS
H. A. RODENHISER

December 21, 1938

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[2] Of this amount $2500.00 is deposited in a savings account at 2%.

Report of the Advertising Manager. The annual income from advertising during the past 7 years has averaged $892.85. The 1938 income of $995.76 is the third highest of the last 7 years, and is $102.91 above the 7-year average.

The total number of advertisements run in 1938 was 172. Of this, 126, or 73.3%, were revenue advertisements. Twenty-one were full-page, 43 were one-half-page, 60 were one-quarter-page, and 12 were one-eighth-page.

Forty-six advertisements occupying 37 pages were non-revenue-producing. These consisted of exchange advertisements with other journals, space occupied by the directory of advertisers, announcements of meetings, and the advertisment of Phytopathological Classics.

During 1938 17 commercial concerns used PHYTOPATHOLOGY as a medium for advertising.

The Advertising Manager wishes to thank the members and officers of the Society for their help and cooperation during his 7 years' service.

R. S. KIRBY

Report of the Editor in Chief. PHYTOPATHOLOGY, Volume 28, 1938, comprises, exclusive of the index, 939 pages (247 pages less than in Volume 27) of printed matter, including illustrations, classified as follows: One hundred articles, 40 phytopathological notes, 3 reports of regional and other meetings, 7 book reviews, 105 abstracts (7 by title only), 196 text figures, 3 plates, and 3 frontispieces. From Jan. 1 to Dec. 31, 1938, 161 manuscripts of articles, notes, reports, and book reviews were submitted for publication in PHYTOPATHOLOGY. Of this number 2 were not accepted, and 16 were returned to their authors for more or less complete revision and condensation. Of those manuscripts received in 1937, but too late for publication in Volume 27, 11 were returned for author revision. In addition to papers published this year, 29 articles, 10 phytopathological notes, and 110 abstracts are now in press. The index for Volume 28, comprising 35 pages, was published in the December number.

The general improvement in the quality of manuscripts, mentioned in the Editor's report of a year ago, has been maintained throughout the current year. Disappointment has been expressed in a few instances because of unsatisfactory reproduction of illustrations. In each such instance, save one, the failure to come up to author expectation has been traceable to poor or indifferent photographs. Too much attention cannot be given to this important detail. Not a little difficulty and delay can be avoided by giving more thought to the composition of full-page or other illustrations. If contributors to our journal will include in such illustration only such photographs or drawings as call for essentially the same reproduction treatment, the results will be more satisfactory.

Attention is here called to the fact that delay in publication can be obviated if, in every instance where a manuscript has been "approved for publication," and so indicated, by the director of the experiment station or by some other responsible official, such approval mean that the manuscript has met the requirements of an editorial committee duly authorized to pass upon manuscripts as a preliminary to substituting them to PHYTOPATHOLOGY.

We are again indebted to Dr. F. V. Rand for his able service in preparing the index of our Journal, and to The Science Press Printing Company for the part it has had in publishing PHYTOPATHOLOGY.

H. B. HUMPHREY

Report of the Manager of Phytopathological Classics for the Fiscal Year December 15, 1937, to December 15, 1938.

Classics No. 1:	On hand 12–15–37	175	
	Sold during year	36	
	On hand 12–15–38		139
Classics No. 2:	On hand 12–15–37	378	
	Sold during year	35	
	On hand 12–15–38		343
Classics No. 3:	On hand 12–15–37	488	
	Sold during year	45	
	On hand 12–15–38		443
Classics No. 4:	On hand 12–15–37	557	
	Sold during year	49	
	On hand 12–15–38		508
Classics No. 5:	On hand 12–15–37	899	
	Sold during year	126	
	On hand 12–15–38		773

Cash balance on hand 12-15-37	$ 24.11	
Receipts during year	279.00	
Total cash income		$303.11
Expenditures during year:		
Postage and express	$ 15.48	
Stationery	3.25	
Advertising	8.50	
Exchange on foreign drafts	.38	
Total expenditures		27.61
Balance on hand December 15, 1938		$276.50
Due on account		$ 7.75

H. H. WHETZEL

Report of the Committee on Necrology. During the calendar year 1938, there have been two deaths of members as follows:

MR. D. C. GEORGE, February 11
MR. F. C. MEIER, July 29

A. G. JOHNSON
M. B. WAITE

Following the reading of the necrology report, the members present stood for a moment in silence in honor of their departed colleagues.

Report of the Committee Representing the Society in Relation to the Union of American Biological Societies and Biological Abstracts. At the Indianapolis meeting, the Society merged the functions of two sets of representatives in a single committee with the Secretary and the Editor in Chief as members *ex officiis*. The Committee's first task was to ascertain the extent to which the members of the Society would be willing either to subscribe for the 1938 volume of Biological Abstracts or to contribute voluntarily as much as $2.00 for its support. Favorable responses, about equally divided, were received from more than 200. These helpful pledges were turned over to Biological Abstracts.

The chairman of the Committee was appointed early in 1938 to the new Board of Trustees of Biological Abstracts, headed by G. W. Hunter, III. This Board decided to publish the 1938 volume only after the money was in sight to meet minimum budget requirements. Not until April was it clear that this was assured by the cooperation of the 5 societies that contributed $2.00 per member, the additional voluntary pledges, and the subsidies granted by some 485 institutions. Under J. E. Flynn, Editor in Chief, publication then proceeded with unprecedented promptness and demonstrated the ability of the necessarily curtailed editorial staff to shorten to a gratifying brief interval the time between the appearance of articles and the printing of the abstracts. The new business manager has also taken hold effectively and Biological Abstracts is reaching the close of the year within the budget.

After serious deliberation, the new Board concluded that in the absence of endowment Biological Abstracts could not hope to survive and serve biological science effectively if forced to depend on subsidy. Believing that by more widely disseminating the benefits of the service and making it indispensable to more individuals and institutions, Biological Abstracts could be placed on a permanently self-sustaining basis, the Board decided, starting with 1939, to abandon the institutional subsidy plan, to charge one price to all, and in addition to the complete edition at $24.00 a year to issue parts comprising closely related subject matter at prices ranging from $4.00 to $9.00.

Of greatest interest to this Society, of course, is Section D—Abstracts of Plant Sciences ($6), comprising plant diseases (including viruses), as well as plant physiology, plant anatomy, systematic botany, agronomy, horticulture, forestry, pharmaceutical botany, paleobotany, etc.

To insure maximum usefulness it also was decided to ask the various biological societies to participate in determining the policies to be followed in their respective subject-matter fields. To make continued operation possible, yet hold prices down, the most rigid economies have been instituted and advantage has been taken of the voluntary abstracting and editorial services of a large number of distinguished biologists without whose loyalty the service could hardly have survived.

It is hoped that the plans for the future of this service, conducted, as it is, on a non-profit basis by biologists for biologists, will appeal to all as so sound and sensible that there will be a rapid increase in the number of institutional and individual subscriptions. Since during the initial year under the new plan the full normal volume of subscriptions cannot be expected, continued contributions from societies and individuals will be most helpful. Every cent thus received goes into the service and it is expected that the increased subscriptions will not only sustain Biological Abstracts but will eventually make possible more complete coverage and more critical abstracting without sacrifice of the promptness deemed essential by the Board.

The Union of American Biological Societies, at its annual Council meeting at Indianapolis, elected E. V. Cowdry, president, G. W. Hunter III, secretary, and D. H. Wenrich, treasurer, for the year 1938. During the year the Union has continued to promote the interests of Biological Abstracts, which was the original *raison de'etre* of the Union. It also has established a Committee on Biological Science Teaching headed by Dr. Oscar Riddle.

In July, 1938, this committee achieved the establishment of the National Association of Biology Teachers, representing the group of more than 20,000 who teach biology in secondary schools. In October, 1938, this Association inaugurated the publication of a journal, the American Biology Teacher. The committee is working to form local units of this national association, which already has attained a membership of about 1500. The Carnegie Foundation for the Advancement of Teaching has granted $10,000 for a 3-year period in support of this work.

The Union is eager to serve the member societies in any matters of interest to them collectively or individually and will welcome suggestions from individuals or officers of these societies.

EDSON
HUMPHREY
KEITT
KIRBY
REDDICK
BARSS (Chairman)

Report of the Committee on Regulatory Work and Foreign Plant Diseases.

1. The members of your Committee have given careful consideration to the federal and state laws in present operation regulating protection from insect pests and plant diseases and agree that, as at present constituted, they have not adequately achieved the purposes for which they were put into force.

2. Your Committee urges that a careful study be made by members of the Society of any proposed modifications of existing quarantine and regulatory laws or services with a view to elucidating their possible effects upon agriculture and forestry from the phytopathological standpoint.

3. The Committee feels that adequate discharge of responsibilities relating to plant protection cannot be achieved by federal or state regulatory agencies without more adequate representation of phytopathological science.

4. Because of the increasing need for current information regarding the prevalence of domestic and foreign plant pathogens we urge that the Office of Mycology and Disease Survey take the necessary steps to bring up to date the very valuable lists published some 20 years ago entitled, Some Common Plant Diseases in the United States by P. J. Anderson and R. J. Haskell, and Foreign Plant Diseases by John A. Stevenson.

5. The Committee desires to call attention to the very effective control of wart disease of potatoes within the state of Pennsylvania and are of the opinion that these leads should be followed up and put into operation without further delay throughout all areas under quarantine for this disease and thus effectively rid the country of these potential sources of infection.

6. The Committee views with alarm the steady increase of virus diseases among many crop plants of the United States and urges most emphatically that the seriousness of the virus situation be more practically recognized and recommends that greater stress be placed upon systematic field inspection services with a view to possible certification of crops essentially reproduced by vegetative propagation, and that, in order to achieve more practical results, we further recommend that greater emphasis be placed upon researches leading to the discovery of simple diagnostic methods of determining virus diseases in the field.

7. The Committee further advises that a new and economically important bacterial disease of potato is present on the North American Continent, which, more than any other disease known, threatens to curtail profitable production of potatoes unless systematically controlled whenever it makes its appearance. It is especially recommended that any State conducting seed potato certification services should allow no tolerance of this disease.

C. R. ORTON, Chairman

Report of the Committee on Extension Work and Relations. The Extension Relations Committee sponsored two activities in 1938: The first was a tour for the purpose of studying the results of research and extension work on the control of peanut diseases in

North Carolina. This tour was made on September 19 and 20. About 30 people went on the tour and represented the agricultural experiment stations and extension services of Virginia, South Carolina, and North Carolina, the United States Department of Agriculture, and various commmercial concerns. The peanut-disease problems given most consideration on the tour were: (1) the control of Cercospora leafspots with various fungicidal dusts and sprays, and (2) the control of root rots by crop rotation and chemical soil treatments.

The second activity was a conference on the subject of "Seed Treatment for Disease Control," which was held on the afternoon of December 28 at the Richmond meeting of the Society. The subject was broken down into 3 sub-topics, and the discussions led by individuals, as follows: (1) "Vegetable Seed Treatment," by J. G. Horsfall; (2) "Cotton Seed Treatment," by S. G. Lehman; and (3) "Cereal Seed Treatment," by R. J. Haskell. Approximately an hour was devoted to the discussion of each sub-topic, and an effort was made to give consideration to both the research and extension phases of each problem. The attendance at this conference was about 100.

The following recommendation was adopted by the Extension Relations Committee at its formal meeting on the evening of December 29, at Richmond:

"The Extension Relations Committee of The American Phytopathological Society recommends that a permanent rotating publicity committee be appointed for the Society for the purpose of furthering plant pathology and acquainting the general public with plant diseases."

LUTHER SHAW,
R. J. HASKELL,
R. S. KIRBY,
W. B. TISDALE,
CHARLES CHUPP.

Report of the Committee on Coordination in Seed-treatment Research. Seed-treatment research has gone forward during the past 3 years. However, the outstanding development in this field during this period is not in the investigations but in the rapid growth of the practice. Some of the reasons for this very rapid growth are recognized easily.

The period was preceded by adequate research showing the beneficial effects of dry seed treatments over the cumbersome wet treatments.

The treatment of small grains with dust fungicides was limited because of the cost of materials. A 75 per cent reduction in cost was a stimulus to the practice of seed treatment at this time.

Extension pathologists seemed to be waiting for this opportunity to develop well-organized programs. These programs centered in traveling machines in such States as Indiana and Illinois, in central treating plants in elevators in Iowa and North Dakota, and in simplified methods of treating by individual farmers in Minnesota.

The pyramiding increases in the use of hybrid seed corn have increased seed treatment of corn, because nearly all hybrid seed corn is treated before it is marketed. In Wisconsin it has a place in the hybrid seed-corn certification program.

The value of sugar-beet-seed treatment has been demonstrated in such an emphatic way as to have resulted in the treatment of 220,000 pounds in Iowa alone in 1938.

The principal contributions in seed-treatment research during the past 3 years are a broader recognition of the value of corn seed treatments in early plantings, the partial protection by seed treatment of corn kernels with broken seed coats, further data on the effect of different periods and conditions of storage on treated seed, and obtaining sufficient data to make recommendations for treatment of sugar beet seed.

Barbak C has replaced Barbak III as a seed-corn dust fungicide. In limited trials it has been somewhat better than the old product.

C. S. REDDY.

Report of the Committee on New Memberships and Subscriptions. The membership committee, through the courtesy of the Secretary, obtained on a series of library-size cards a list of all the present membership in the United States and Canada. These cards were sent, respectively, with a letter to a leader in each State and Province with the request that the leader secure as many new members as possible from those eligible in his district. In addition, a notice was included in PHYTOPATHOLOGY and a form letter was sent to each member along with the official ballot, through the courtesy of the Secretary. Early in December a follow-up letter was sent to each State and Province leader.

A. J. RIKER.

Report of the Committee on the Standardization of Experimental Spraying Technique. The above temporary committee appointed by President Anderson on November 1, 1938, to "canvass the possibility of securing (1) standard methods of testing fungicides and (2) adequate laws for controlling spray materials offered for sale," realizing the importance and complexity of this problem recommends that a permanent standing Committee on the Standardization of Fungicidal Tests be appointed by the Council of The American Phytopathological Society.

The duties of this committee shall be to investigate and recommend for official approval:

A. Standardized laboratory methods for testing fungicides.

B. Experimental designs for laboratory and field tests and statistical methods for evaluating the results therefrom.

C. Adequate laws controlling the declaration of ingredients of commercial fungicides.

The following program of investigation is proposed:

Under A—

1. A study of methods of testing protective fungicides in the laboratory to determine the most suitable for standardization.
2. A tentative method or methods having been determined, selected fungicides should be tested by various laboratories and the method found satisfactory before it is recommended as official.
3. Publish a description of the recommended methods.
4. Canvass the possibility and desirability of a central testing laboratory where tests may be made for interested persons and commercial firms.
5. Investigate the possibilities of a Bordeaux coefficient for evaluating copper sprays and dusts.
6. Investigate the possibilities of a standard laboratory or greenhouse method of testing spray injury.

Under B—

1. Determine suitable experimental designs for laboratory and for field tests. Likewise, statistical methods for evaluating the results of such experiments.
2. Publish the recommended designs and statistical methods.
3. Arrange for the field testing at different localities of selected fungicides previously tested in the laboratory and correlate the results of laboratory and field tests.

Under C—

1. Study the present fungicide laws and be prepared to make recommendations for the further control of fungicides offered for sale.
2. Consider a system of registration whereby all manufacturers offering fungicides for sale should be required to register their products and to declare the nature and content of both active and inert ingredients.
3. Consider the desirability of including within the scope of our fungicide and insecticide laws, products designed to be used against virus diseases, nematodes, non-parasitic diseases, etc.

It is believed that, while the term fungicidal should be interpreted in its broadest sense, the activities of the committee, for the present, should be restricted to the above proposals.

It also is believed that the aims of the committee may be furthered by the appointment of appropriate sub-committees and further that the committee should keep informed of improvements and trends so that it is prepared to recommend modifications of any "official" methods.

W. H. MARTIN,
J. W. ROBERTS,
H. C. YOUNG,
K. J. KADOW,
S. E. A. MCCALLAN, Chairman.

Report of the Advisory Committee on Society Activities and Programs. Your temporary committee recommends the constitution of a standing Advisory Committee on Program. It is the sense of your committee that the annual programs of the Society are good and that they are well received by the membership. It is not contemplated that the proposed committee should take over any of the responsibilities or powers of the Council, the Program Committee, or other committees now constituted; but, rather, that it should serve in an advisory relationship, with special reference to matters of program policy and the larger aspects of program planning. It is recommended that the composition of the proposed committee be left to the discretion of the Council, with the suggestion that the principle of rotating membership be followed.

Your committee recommends that it be discharged.

H. P. BARSS,
G. H. COONS,
M. W. GARDNER,
H. T. GÜSSOW,
L. M. MASSEY,
I. E. MELHUS,
E. C. STAKMAN,
G. W. KEITT, Chairman.

Report of the Committee on Technical Words. It should be clearly understood that we are not deluding ourselves with the dream that we can standardise usage or induce all plant pathologists to use all or even most of the words on our list. Experience with rules of nomenclature and committees on common names has clearly shown that our associates will use the terms they choose in the way they choose, regardless of rules or lists.

In attempting to carry out the instructions of the Society following suggestions made at the Amsterdam Congress, we feel that we may reasonably hope to prepare a list of terms usable in the publications of our profession, so defined that they may be generally understood internationally, and so generally available by international publication that any person may use them without defining his terms. Even this constitutes a difficult job. The question has been frequently raised as to whether it is ever possible to explain words with words. To that end we are asking for additional lists of words with definitions, to add to the several excellent ones already received, before we undertake the completion of a list to submit to the Society. Anyone who takes the trouble to prepare such a list will find that the attempt is worthwhile, even though not all the words are finally included. As Graham Wallas remarks, "The extension of association produced by the search for the right word in which to express a thought may often result in stimulating a new and better thought."

JESSIE I. WOOD,
NEIL E. STEVENS,
DONALD REDDICK, Chairman.

Report of the Committee on International Plant Pathology. Dr. Jacob Eriksson advocated at every opportunity the establishment of an institute of research for plant pathological problems of international importance. One of his last appeals appeared in PHYTOPATHOLOGY V. 5, under the title "International Phytopathologic Collaboration: Work Begun in Europe—Will It Be Prosecuted in America." A bibliography of thirteen titles is appended, ten of them referring to Eriksson's own writings.

In some instances it is evident that Dr. Eriksson was thinking in terms of a physical plant and a staff of scientists. The members of your committee are of the opinion that the idea of a physical plant with an endowment, a director and a staff of experts is wholly impracticable. In this changing world, endowments are not very stable, even assuming that an endowment could be secured at all, directors are not always competent, and it is impossible to assemble a sufficient staff of leaders because the real leader within a given field of investigation is likely to change from year to year if, indeed, not from month to month. Furthermore, any particular locality in the world would be the most suitable place for the pursuit of relatively few of the researches that have to be made.

We feel that our Society has made progress toward the goal pointed out by Dr. Eriksson and that while the progress has been painfully slow, the groundwork has been laid for effective cooperation in the solution of problems of international purport. We sponsored a section for Phytopathology in the International Botanical Congress held in Ithaca in 1926; we made sure at Cambridge in 1930 of sectional autonomy in future congresses; we entered the International Union of Biological Sciences and helped secure an autonomous status, within the limitations of the very catholic statutes of the Union itself, at Amsterdam in 1935; we are making preparations to participate in the deliberations of the Council at Stockholm. It is easy to believe that we shall be able to exert an influence there in direct proportion to the time and energy we are willing to put into the endeavor.

In the opinion of your committee an "institute" now exists; it is in fact the subsection for Phytopathology of the International Union of Biological Sciences. Officers of the subsection were authorized to execute any mandates of the Botanical Congress and to act as an interim committee for urgent phytopathological needs. These officers are elected in a thoroughly democratic way, they are not eligible to immediate reelection, they constitute a steadily rejuvenated board of directors of the Institute. If so instructed the officers could, without much difficulty, call for a list of problems considered to be of international consequence, formulate some or all of them, and publish the list. The officers also could canvass the research laboratories of the world and find out and publish the names and places where post-doctorate scholars are welcome and where facilities are available and favorable for the pursuit of specific problems. At the present time such printing as is involved in this phase of the enterprise is being actively solicited by the editor of CHRONICA BOTANICA. Certain other incidental and clerical expenses would be involved. It is possible that a part of the "dues" regularly paid by the adhering countries could be allocated to this purpose.

By this plan the officers and staff of the Institute would comprise the research pathologists of the world; the program of work of the Institute would embrace the whole realm of plant pathology; the laboratories, libraries, etc., would consist of the apparatus, equipment, books and facilities of all of the pathological institutes of the whole world.

There remains the very important item of financing the researchers. On first thought this appears to be the crux of the whole matter. Upon reflection, however, it is not even certain that a magnificent endowment for the purpose would be the correct answer, even assuming that there were any practicable way to establish such an endowment. The management of funds requires a different type of mind from that possessed by persons engaged in plant pathological research. Many of us have witnessed at home or abroad the dwindling of income from, or the complete disappearance, of trust funds. For the moment, at any rate, we must seek other means of financing research. A great many institutions and foundations in the world finance research and in many cases the selection of researchers is so unrestricted that grants in aid of phytopathological research might await only the formulation of a plan of work sufficiently comprehensive to justify the expenditure.

No investigation of the possible sources of funds has been made, and there is no assurance that plant pathology could be financed by the specific organizations about to be mentioned but they at least represent the type of institution that might be interested in applications for grants of funds for research. The Committee on Intellectual Cooperation of the League of Nations, British Commonwealth Fund, Belgian-American Educational Foundation, German Notgemeinschaft der deutschen Wissenschaft, U. S. National Research Council, Guggenheim Foundation, the recently established Office in the U. S. Department of State for the encouragement of cultural relations among the American nations, traveling fellowships of universities, academies, institutes, museums, and the like. The National Research Council has compiled a list of fellowships and scholarships that are available in the United States. Such lists probably exist in other countries, or, at least could be got, and from such lists it should be relatively easy to make up a new list of those available to phytopathologists.

NEIL STEVENS,
DONALD REDDICK.

Report of the Committee on Internationalizing Physiologic Races. The American Phytopathological Society strongly urges that the same general principles of priority that guide procedures in taxonomy be used also in the designation of races of rust fungi, to this extent: that a uniform system of numbering be used and that new numbers be not assigned to races already described under another number. The sole object of this recommendation is to avoid confusion resulting from the designation of the same race by several different numbers, as is now often the case.

E. C. STAKMAN,
A. G. NEWHALL,
H. B. HUMPHREY, Chairman.

Report of the Resolutions Committee. 1. RESOLVED that The American Phytopathological Society express its appreciation to the A. A. A. S. committees responsible for the arrangements that have contributed so effectively to the success of the 1938 meeting in Richmond.

2. RESOLVED that The American Phytopathological Society convey to the management of the Jefferson Hotel expression of gratitude for the courteous and efficient service extended to the members attending the thirtieth annual meeting.

3. RESOLVED that, on behalf of The American Phytopathological Society, we express our appreciation to the various local agencies and committees for their many courtesies and most efficient services, particularly to the Hon. Mayor J. Fulmer Bright, and his efficient police force; to Mr. Foley F. Smith and his committee on housing; to Mr. Wm. Clift and his committee on transportation, registration, and information; to Mr. Rodney C. Berry and his committee on meeting places and projection lanterns; to the Second Baptist Church for permitting the use of their building for our meetings; and to the Richmond Chamber of Commerce for its cooperation.

4. RESOLVED that, on behalf of the members of our Society, we express to our officers and committee members our deep appreciation for their untiring efforts in furthering the best interests of our Society and in advancing the many-sided science of plant pathology.

5. RESOLVED, that the members of our Society in attendance at this our thirtieth annual meeting, express to Dr. F. V. Rand and his committee on entertainment, and to the others who assisted in various ways, our sincere appreciation for the excellent program of entertainment at our annual dinner.

6. RESOLVED that, on behalf of The American Phytopathological Society, we convey to Mrs. Agnes E. Meier our sincere sympathy and very deep sense of loss in the tragic death of Fred C. Meier, her husband and our former Secretary-treasurer and former Vice-president. He has been so constructively active in our Society for so many years that we sorely miss his ever cheerful companionship and helpful conferences. He will long be remembered most kindly.

W. G. NEWHALL,
T. F. MANNS,
A. G. JOHNSON, Chairman.

Report of the Representative with the International Union of Biological Sciences. As was aptly put by Dr. Reddick in 1937, the value of the International Union and particularly the subsections on phytopathology to our Society and to North America depends on what use we make of it. In order to make effective use of it, proposals should be submitted at least six months before the convention, which means they should be written, circulated and thoroughly discussed, and ratified by our Society in session either at the Richmond or Columbus meetings (1938 and 1939), going to the secretary, Johanna Westerdijk by February, 1940.

At present writing, December, 1938, no definite proposals have been made that the writer is aware of, though some very likely will be soon.

At this time it may be well for us to ask ourselves some such questions as the following:

1. Can we agree on definitions of terms related to immunology so that among ourselves we present a satisfactory and united opinion?
2. Are there other phytopathological terms the international usage of which we can help to clarify by agreeing on definitions?
3. Do we wish to express our approval or appreciation of the growing tendency for foreign scientists to include English summaries in their papers?
4. Are we ready to press for adoption of the report of the International Committee for Description and Nomenclature of Plant Viruses?
5. Have we any concrete proposals to make that might help bring to realization the dream of Eriksson's International Phytopathological Institute? Would this be promoted by strengthening the Subsection of Phytopathology, raising it to a more permanent level with continuation powers to exercise between international meetings?
6. Is it time we made an effort to obtain financial aid that would permit us, in conjunction with other botanical organizations, to give an invitation to the International Union of Biological Sciences to hold a meeting in the United States?
7. Are there any international quarantine principles that we should clarify or reiterate or on which we should take a stand?
8. Are there any restrictions, or prohibitions, we should advocate in regard to movement of diseased seed stocks from commercial into seed channels?
9. Steadily growing interest in the South American Republics suggests that it may be a good time to explore the possibilities of Pan-American phytopathology. Many South American students have received part of their education in the United States. Further interchange of scholars is contemplated by the newly established office for the "promotion of cultural relations" under the U. S. Department of State. A special committee of this Society might properly investigate the field and report back at an appropriate time.

A. G. NEWHALL.

Report of the Representative of the Tropical Research Foundation. The Tropical Plant Research Foundation continues its corporate organization intact, as it has since Dr. Orton's death. The Executive Committee consists of R. A. Harper, Wm. Crocker, and L. R. Jones, with Dr. Crocker "Acting Director of Scientific Work." The only plans for any significant development during the past two years were under the leadership of Fred Meier, with the idea of correlation with expected federal developments. Since these were conceived as under his personal leadership, we may here report this as another great loss in potential developments.

L. R. JONES.

ACTION BY THE SOCIETY AT THE 1938 RICHMOND MEETING

Elections and Appointments. The appointments made, as provided by the Constitution, by the President or the Council since the previous meeting were approved by the Society in business session. The election committee opened and counted the ballots, and the results were announced to the Society. The names of those elected and appointed appear earlier in this report in the list of officers, representatives, and committees. Seventy-nine applicants were elected to membership, including the transfer of the life membership of F. C. Meier to his widow, Mrs. Agnes E. Meier.

Following the announcement of the election of H. W. Anderson as councilor for two years, thereby leaving open the one-year term on the Council of the retiring president, the Council appointed E. B. Lambert as councilor for 1939.

The Society confirmed the Council's appointment of the following new members to the Editorial Board of PHYTOPATHOLOGY: Neil E. Stevens, H. R. Rosen, F. J. Stevenson, and A. J. Ullstrup.

Reports of Officers, Representatives, and Committees. The reports for the year 1938, as presented on previous pages, were read and accepted.

Amendment to the Constitution. After being notified in October, the members present at the business meeting on December 27, 1938, voted to amend sentences 2 and 3 of Article 5 of the Constitution to read as follows: "The President and Vice-President will serve for one year or until their successors are elected, and the Council shall fill any vacancies occurring between annual meetings. The Secretary and Treasurer shall be appointed by the Council for a term of 3 years and the Council shall fill any vacancy occurring between annual meetings.''

Recommendation Regarding the Standing Rule on Crop Protection Institute. The Council made the following recommendation, which was adopted by the Society: ''The Council recommends that Standing Rule 10 reading, 'Representatives on the Board of Governors of the Crop Protection Institute shall be provided for by 3 trustees, with 3-year terms, one selected each year, these trustees to be chosen by the Council,' be struck out.''

Recommendation Regarding Abstracts. The following recommendations of the temporary activities committee concerning abstracts of papers to be presented at annual meetings were adopted by the Society:

''Members who plan to present papers at the annual meeting must submit abstracts which shall be received by the Secretary on or before *November 1st.*

''*Abstracts.* Each member is limited to 2 papers on which he may appear as sole, senior, or junior author. Members are requested not to submit abstracts unless they expect to attend the meeting. These abstracts shall be clear and concise, and shall contain no tabular data, and shall not exceed 200 words in length. They shall include only statements of fact, unpublished information, and the directly derived conclusions or hypotheses. Reports of progress, or of disease occurrences, or of routine tests of ordinary control measures, are not desired, unless new and significant developments are clearly indicated. Abstracts are to be reviewed by a committee appointed annually by the Editor in Chief of PHYTOPATHOLOGY, and this committee is directed to return to authors for revision such abstracts as fail to meet the above requirements.

''*Papers.* Members are requested to limit presentation time to 5 to 10 minutes. The maximum time allowed for other than invitation papers will be 15 minutes. Complicated tables or graphs should not be shown.''

This action, therefore, amends Standing Rule 3.

Clearing House for Positions. The Council recommended the desirability of the Secretary acting as a clearing house with whom those desiring positions may file their qualifications, and to whom those desiring to hire pathologists may apply for a list of members who would be available. The Society confirmed this recommendation.

Enlargement of Scope of Seed Treatment Committee. The Society confirmed the Council's recommendation that the scope of the standing committee on coordination in seed treatment research be enlarged to include both vegetables and cereals.

Committee on Standardization of Fungicidal Tests. The Society confirmed the Council's recommendation that the temporary committee on Standardization of Spraying Technique be made a standing committee, Standardization of Fungicidal Tests.

Committee on Society Activities and Programs. The Society confirmed the Council's recommendation that the temporary committee on Society Activities and Programs be made a standing committee.

Biological Smoker. The Society voted a contribution of $10 for the Biologists' Smoker arranged by the American Society of Naturalists at Richmond.

Cumulative Index of PHYTOPATHOLOGY. Upon recommendation of the Council, it was voted that the President-elect appoint a committee to determine the cost and feasibility of publishing a cumulative index of PHYTOPATHOLOGY.

Summer Meeting. The Society, on Council recommendation, voted to hold a summer meeting at Milwaukee, Wisconsin, in connection with the A. A. A. S. summer meeting, June 19–24, 1939. This meeting will be held under the auspices of the Upper-Mississippi Valley plant pathologists.

Invitation Paper. The Society voted that the Editor publish the invitation paper of C. W. Bennett, presented at the Richmond meeting.

Editorial Policy. The Society voted that the Editor in Chief be allowed to use his discretion in accepting papers for publication in PHYTOPATHOLOGY.

Statement of Policy. The Society voted to accept a statement of policy to be followed in connection with international affiliations in the field of organized plant pathology, as presented by Dr. Donald Reddick:

''The American Phytopathological Society was conceived on a broad basis, has admitted pathologists from all parts of the world to membership. The Society fostered a program of papers in a section of the International Botanical Congress at Ithaca and thereafter formulated plans for an international organization of plant pathologists. It subsequently became apparent that a place could be made for the plant pathologists within the structure of the International Union of Biological Sciences. The statutes of the Union are so catholic that within very broad limitations the pathologists can enjoy practically complete autonomy. The machinery exists here for international cooperation as among ourselves as well as with other divisions of biology. It is, therefore, the declared intention of our Society to lend its undivided support to the Union so long as it serves the purpose of an international forum and promotes international cooperation and good will.''

Vote of Appreciation to International Committee on Plant Viruses. The Society confirmed the following resolution of the Virus Committee of The American Phytopathological Society:

RESOLVED, that The American Phytopathological Society express to the International Committee on Plant Viruses appreciation of the work it has done and recommend that the Committee continue its efforts to establish an acceptable system of virus nomenclature.

FRED CAMPBELL MEIER

April 5, 1893—July 29, 1938

Fred Campbell Meier was graduated from Harvard University, with the degree of Bachelor of Science, in 1916 and was granted the degree of Master of Arts by the same institution in 1917.

From 1916 to 1918 Mr. Meier was an Austin Teaching Fellow at Harvard University and from 1918 to the time of his tragic death he served the U. S. Department of Agriculture in various capacities as follows: Assistant Pathologist, Bureau of Plant Industry, 1918–19; Pathologist, Bureau of Agricultural Economics, 1919–21; Pathologist, Bureau of Plant Industry, 1921–22; Extension Pathologist, Office of Cooperative Extension Work, 1922–30; Principal Pathologist in Charge of Barberry Eradication, Bureau of Plant Industry, 1930–34; Senior Agriculturist, Extension Service, 1934–July 9, 1938; and Principal Agriculturist, in charge of Aerobiology, Bureau of Plant Industry, July 10, 1938, to the time of his death.

In addition to being a sustaining life member of The American Phytopathological Society, Mr. Meier served this Society as Secretary-Treasurer and Business Manager of PHYTOPATHOLOGY from 1929 to 1934, and as Vice-President in 1935. He contributed much toward the organization of the Tropical Plant Research Foundation and, at the time of his death, was Chairman of the Committee on Aerobiology of the National Research Council.

Mr. Meier's all-absorbing interest in science was in its relation to human welfare. He possessed unusual initiative and resourcefulness as well as remarkable ability for "blazing new trails." His pleasing personality combined radiant enthusiasm with good will and consideration for others. This won for him a wide circle of friends. He was ever alert and eager to serve where he could contribute most.

DOROTH CHAUNCEY GEORGE

August 15, 1887—February 11, 1938

Doroth Chauncey George was graduated from Washington State College with the degree of Bachelor of Science in 1912, and in 1916 he received the degree of Master of Science from the same institution.

From 1912 to 1917 he served as Assistant Plant Pathologist at the Washington Agricultural Experiment Station. From 1917 to the time of his death he served as plant pathologist, and from 1931 to time of his death, also as State Entomologist, for the Arizona Commission of Agriculture and Horticulture.

Mr. George was a man of sterling character and high ideals. Throughout his career he applied himself unselfishly to the interests of those in whose service he was employed.

ERRATA, VOLUME 28

Page 58, 3rd line from bottom, *read* grown to maturity *for* grown on maturity.

Page 123, 2nd line from bottom, *read Myzus pelargonii* (Sulz.) *for Myzus solani* (Kalt.).

Page 139, line 19 and elsewhere, *read Turritus for Turritis.*

Page 142, citation 3, *read* American *for* African.

Page 143, line 30 and page 145, line 5 from bottom, *read Ustilago for Ustilaga.*

Page 144, line 2 of paragraph 1, *read tritici for triticti.*

Page 174, table 1, column 4, *read* 1859 *for* 1858.

Page 200, lines 7 and 12, *read* Peltier and Samson *for* Peltier, King, and Samson.

Page 319, line 10, *read* 36 *for* 30.

Page 319, line 23, *read* 1–300,000 *for* 1–200,000.

Page 330, table 1, column 3, line 1, *read* C^b *for* C^a.

Page 331, table 2, column 4, last line, *read* 10^{-5} *for* 10^{-8}. Column 5, last line, *read* 10^{-8} *for* 10^{-5}.

Page 356, line 30, *read* 29 *for* 19.

Page 375, line 1, *omit* (Fig. 1).

Page 401, citation 17, *read* **20** *for* **22**.

Page 434, line 6, *read* decaying *for* decay.

Page 495, table 1, column 5, *read* + *for* ×.

Page 680, line 21, *read* Type on Turkish *for* Type of Turkish.

Page 757, line 8, *read* the fig. *for* figure 1.

Page 868, line 32, *read* table 4 *for* table 3.

SEVERAL SPECIES OF PYTHIUM CAUSING BLOSSOM-END ROT OF WATERMELONS

CHARLES DRECHSLER

(Accepted for publication January 23, 1939)

A field decay of watermelon, *Citrullus vulgaris* Schrad., fruits resulting from invasion by a parasitic species of *Pythium* was observed by Nelson[1] in Michigan during the summer of 1916. According to brief reports by Coons (8) and by Coons and Nelson (9) affected melons, whether ripe or immature, showed blackened withered spots at the blossom end. While in some instances only a blemish about 75 mm. in diameter resulted from an infection, in other instances the fruit was wholly involved. On artificial inoculation the causal organism obtained in pure culture and held to be probably identical with *P. debaryanum* Hesse, was found to produce decay in healthy watermelons, and to bring about a leaky condition in both muskmelon, *Cucumis melo* L., and cucumber, *C. sativus* L., fruits, though not in potato, *Solanum tuberosum* L., tubers.

Some years later in a brief abstract (13) I recorded a destructive outbreak of two types of blossom-end decay that had occurred in a watermelon field on Arlington Experiment Farm, Arlington, Virginia, during the summer of 1922. Of these types of decay, one, manifested externally by chocolate-brown or bluish-brown discoloration, was held referable to a new species of *Pythium* whose spiny oogonia were commonly found fertilized by 1 to 4 branch antheridia; the other, manifested externally by lighter brownish discoloration, being attributed to *P. debaryanum*. Subsequently, in a consideration mainly of the cottony leak of cucumbers, 2 species of *Pythium* were mentioned (14, p. 1037, 1041) as being responsible for chocolate-brown blossom-end rot of watermelons: one of the species producing mulberry-like zoosporangia; the other giving rise to zoosporangia most often consisting individually of a subspherical part together with an adjacent portion of one or both hyphal elements between which it is intercalated. The lighter colored or buff blossom-end decay received mention (14, p. 1038) as a field trouble apparently widely distributed in the Middle Atlantic States, and there caused for the most part by the same fungus as that most frequently causing cottony leak of cucumbers,—the widely familiar fungus, which, though often cited under the binomial *P. aphanidermatum* (Eds.) Fitzp., would seem, on grounds elsewhere (18) set forth, more correctly designable as *P. butleri* Subr.

The two spiny parasites causing dark-brown blossom-end rot that were provisionally distinguished in 1925 by their very different zoosporangia I described in 1930, along with 13 congeneric forms, as species new to science (17); the fungus with moriform zoosporangia being presented under the binomial *Pythium periplocum,* that with the modified subspherical zoosporangia under the binomial *P. acanthicum.* Apart from these two species, both made known only in their pathogenic connection with diseased watermelons, I attributed decay of watermelon fruits in Florida and Georgia to *P. myriotylum,* also a species then newly described, which, however, like *P. butleri,* was recognized besides as a parasite on various other host plants.

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[1] Nelson, R. A field rot of watermelon caused by *Pythium*. Program 11th Ann. Meet. Bot. Soc. of Amer. held in New York, Dec., 1916, Pub. 66. Short statement of content of this paper, given in footnote by Coons and Nelson (9).

Walker and Weber (30), in an account of watermelon diseases of Florida published in 1931, cited *Pythium debaryanum* and *P. aphanidermatum* among the fungi associated with a group of abnormalities they discussed as blossom-end rot. Kheswalla (22), 5 years later, referred a malodorous rot of watermelons in Baluchistan to *P. aphanidermatum.* To the same species was ascribed in 1937 a watery soft rot found affecting watermelons in the Salt River Valley of Arizona (5). Ramsey, Wiant, and Link (26) in a recent treatise on market diseases of fruits and vegetables, enumerate *P. acanthicum, P. aphanidermatum, P. artotrogus* de Bary, *P. myriotylum* and *P. periplocum* among the organisms causing decay of watermelon fruits.

Although several species of *Pythium* have thus been recorded in the literature as agents causing blossom-end rot of watermelons, these do not comprise all the members of the genus occurring in such parasitic relationship. In some hundreds of cultures that, as opportunity offered during the last 17 seasons, I have isolated from affected melons in different regions, at least 4 other species are to be recognized: *P. ultimum* Trow, *P. irregulare* Buism., *P. mammillatum* Meurs, and *P. helicoides* Drechsl. The first 3 of these additional species have become more or less widely known from their association with damping-off and rootlet decay of various crop plants; and may, therefore, more appropriately be discussed later in connection with a few other forms of similar pathogenic tendencies. For that matter, the less familiar *P. helicoides* also is known to occur on subterranean parts of a crop plant, its original description (17) having been based on cultures isolated from affected roots of the common bean, *Phaseolus vulgaris* L. Indeed, this rather rare fungus has been obtained almost as frequently from diseased roots as from decaying watermelons; but, whereas the few instances of discoloration in the small underground structures have supplied only meager evidence of harmful parasitism, the instances of extensive decay in the massive fruits have, despite their small number, provided an impressive display of destructiveness. Thus, similarity in pathogenic behavior rather than taxonomic kinship brings *P. helicoides* together with the two distinctive blossom-end parasites *P. acanthicum* and *P. periplocum* for such descriptive treatment as may be necessary properly to supplement the earlier diagnoses.

The usual inception of *Pythium* decay in watermelons as a well-marked blossom-end rot finds an explanation mainly in the anatomical structure of the fruit itself. The unbroken epicarp of the berry appears highly efficient in excluding invasion by any species of *Pythium.* Such invasion does not ordinarily ensue, even when the epicarp, together with the chlorophyll-bearing hypoderm immediately underneath (1), is removed over considerable areas through mechanical scraping, or through the feeding of superficially gnawing insects; the very firm stone-cell layer then exposed offering a barrier apparently not easily transgressed by many fungi, notwithstanding the conspicuous freedom with which it is penetrated by the widespread anthracnose organism, *Colletotrichum lagenarium* (Pass.) Ell. and Hals. Deeper wounds, whereby the stone-cell layer is interrupted, usually permit entrance by species of *Pythium,* as also of other fungi, including, for example, *Corticium vagum* B. and C., often encountered in blossom-end rot, and the one or possibly several species of *Diplodia* more familiarly associated with stem-end rot.

Owing apparently to imperfect scarring of the epicarp and incomplete closure of the stone-cell layer at the attachment of the withered floral remnants, the berry is inadequately protected against fungus invasion over a minute apical region. Under normal conditions species of *Pythium* are unable to gain entrance into uninjured melons except by the narrow avenue of insufficiently indurated tissue directly underneath the floral scar. The means by which the parasite gains its initial foothold can only be surmised. In instances where a melon decaying from the distal end is found with its tip directed downward and resting on the ground, it is not difficult to presume that infection took place by growth of hyphae from the soil into the floral remnants and thence through the vulnerable gap in the stone-cell layer. Most infected fruits, however, are found in approximately horizontal positions, or are otherwise disposed in a manner little suggestive of earlier contact between blossom end and ground. In these more numerous instances of infection, it may be conjectured that conidia or oospores of the invading parasite reached the floral scar mainly in particles of soil lifted by winds or splashed upward by rains; germination of the adhering reproductive bodies presumptively having taken place later on when a small quantity of water had remained desposited for a sufficiently long time during periods of dewy, foggy, or rainy weather.

Manifestly, in dry seasons and to a considerable degree in seasons of moderate, well distributed precipitation, the circumstances attending such infection might reasonably be expected to favor organisms with spores not only highly resistant to the rather severe desiccation to which the surface soil of watermelon fields is ordinarily exposed, but also given to especially prompt germination when water becomes available, even in small amounts, for a relatively short period. And, indeed, the one species of *Pythium* generally predominant in the causation of blossom-end rot, *P. acanthicum,* is conspicuous for the unusually prompt germination of its adequately resistant oospores. Under somewhat unusual weather conditions, other species may come to predominate locally; as notably, for example, when *P. butleri* gains a destructive ascendency in Maryland and Virginia during or immediately following spells of excessively hot, moist weather. High temperatures combined with ample moisture appear very important also for the development of *P. myriotylum;* this parasite evidently being limited in the United States to a southern distribution because of its exacting thermal requirements.

Once having gained a foothold the progress of *Pythium acanthicum, P. periplocum*, or *P. helicoides* through an invaded watermelon fruit is marked externally in an advance of chocolate-brown, dark-brown, or bluish-brown discoloration beginning at the blossom end and proceeding toward the stem end. On cutting open invaded specimens, affected parts are easily distinguished from healthy parts by their brownish discoloration and their softened watery texture. The outer rind often presents a watersoaked appearance as if pickled; yet, owing to its sturdy cellular structure, it usually retains some noticeable firmness, while the pulp or inner mesocarp becomes exceedingly flaccid, and on slight pressure gives off its dilute sepia juice in copious quantity. Under a microscope the large-celled tissues of the inner mesocarp are seen to be abundantly permeated by mycelial hyphae that course haphazardly in all directions with little evident regard for the presence of the thin cell membranes. The thickened cell walls of the middle mesocarp and outer mesocarp for the most part constrain passage of the hyphal filaments through their pits; resistance to mycelial extension, therefore, becoming apparent especially in the harder small-celled portions of the outer mesocarp.

At moderate temperatures and in the absence of too severe bacterial contamination, progress of an invading *Pythium* mycelium usually continues until the affected watermelon has been wholly traversed. The somewhat dry, blackish, superficial lesions that ordinarily do not develop further than blossom-end blemishes have in my experience consistently failed to yield any cultures of *Pythium;* and must accordingly be held referable to other causes, despite some writings tending to assimilate them to Pythium decay. Nor, again, has it been possible to ascertain any connection between malformation of watermelon fruit and susceptibility to blossom-end rot of any kind attributable either to species of *Pythium* or to the biologically similar if taxonomically remote *Corticium vagum.*

Like other cucurbitaceous fruits, watermelons, when invaded by species of *Pythium,* including those herein considered, give off a distinctive marshy odor due directly to the action of the mycelium rather than to activity of the bacteria following its advance through the succulent host tissues. Multiplication of these bacteria, however, soon brings about unmistakable putrefaction and gradual disintegration, first in the portion of the melon earliest affected, then throughout the massive berry. Worms, insects and other forms of animal life usually participate diligently in the final uninviting stages of decomposition; although when very young fruits without succulent pulp are invaded, especially in cooler weather, by any of the fungi causing dark brown blossom-end rot, saprophytic molds may intervene more conspicuously, to help destroy the somewhat mummified structures in a slower and less unsavory manner.

As might be expected, all the various species of *Pythium* isolated from tissues affected with blossom-end rot readily produce decay on being inoculated into healthy watermelons by incisions through the stone-cell layer. Whether such experimental results can properly be interpreted as proof of pathogenicity, except in a limited sense, appears doubtful, since, through similar procedure, decay of watermelons is likewise induced by numerous species of *Pythium* that hitherto have never been found affecting this fruit under natural conditions. Of these numerous forms one is given comparative treatment herein, which, along with *P. acanthicum* and *P. periplocum,* was referred to in an earlier paper (14, p. 1041, 1042) as a species incapable of causing decay in cucumber fruit, even after being introduced into incisions through the epicarp.

In most seasons and in most of our watermelon-growing sections, the losses due to blossom-end rot caused by species of *Pythium* would seem not serious enough to justify special control measures. Yet, the rather substantial losses prone to occur during wet years in some parts of the Middle Atlantic States, especially in fields intended to be harvested late in August or in September, suggest that preventive measures might at times be profitably undertaken here. While no experimental work has been done on means of control, it may reasonably be presumed that the fungicidal paste recommended by Orton and Meier (25) for prevention of stem-end rot could be employed advantageously also as a prophylactic against blossom-end decay. One application of the adhesive preparation over the minute area covered by the flower scar—an operation requiring little labor or material—should ordinarily suffice to forestall invasion by any species of *Pythium* or by *Corticium vagum.* On the occasion of such treatment, melons found with their apical ends in contact with the ground could conveniently be shifted into positions less favorable for attack by parasitic fungi.

PYTHIUM ACANTHICUM

In the course of the present studies *Pythium acanthicum* has been encountered more frequently as the cause of blossom-end rot of watermelons (Fig. 1, A, B, C) than all its congeners taken together. This general predominance came to light no less clearly where blossom-end rot occurred only in negligible quantity as, for example, in fields near Kennett, Missouri, and Decker, Indiana, that on inspection late in August, 1924, showed only 1 or 2 affected specimens to an acre, than where the disease was highly destructive, as in some fields near Annapolis, Maryland, that late in September, 1924, showed more than a fourth of the crop being ruined by the decay. The fungus has always been found in greater or smaller quantity whenever watermelon fields with maturing fruit have been surveyed for the presence of blossom-end rot. It has been identified in numerous cultures isolated from material collected near Williamsburg, Maryland, Diamond Springs and Smithfield, Virginia, early in August, 1923; near Gainesville, Leesburg, and Bradenton, Florida, and Thomasville, Georgia, early in June, 1925; near La Fayette, Indiana, late in August, 1928; near Salisbury, Maryland, late in August, 1938; and near Beltsville, Maryland, late in September, 1938. Moreover, a few cultures of the parasite were obtained from rather dry, shallow, cankered lesions that appeared on living watermelon vines at Arlington Ex-

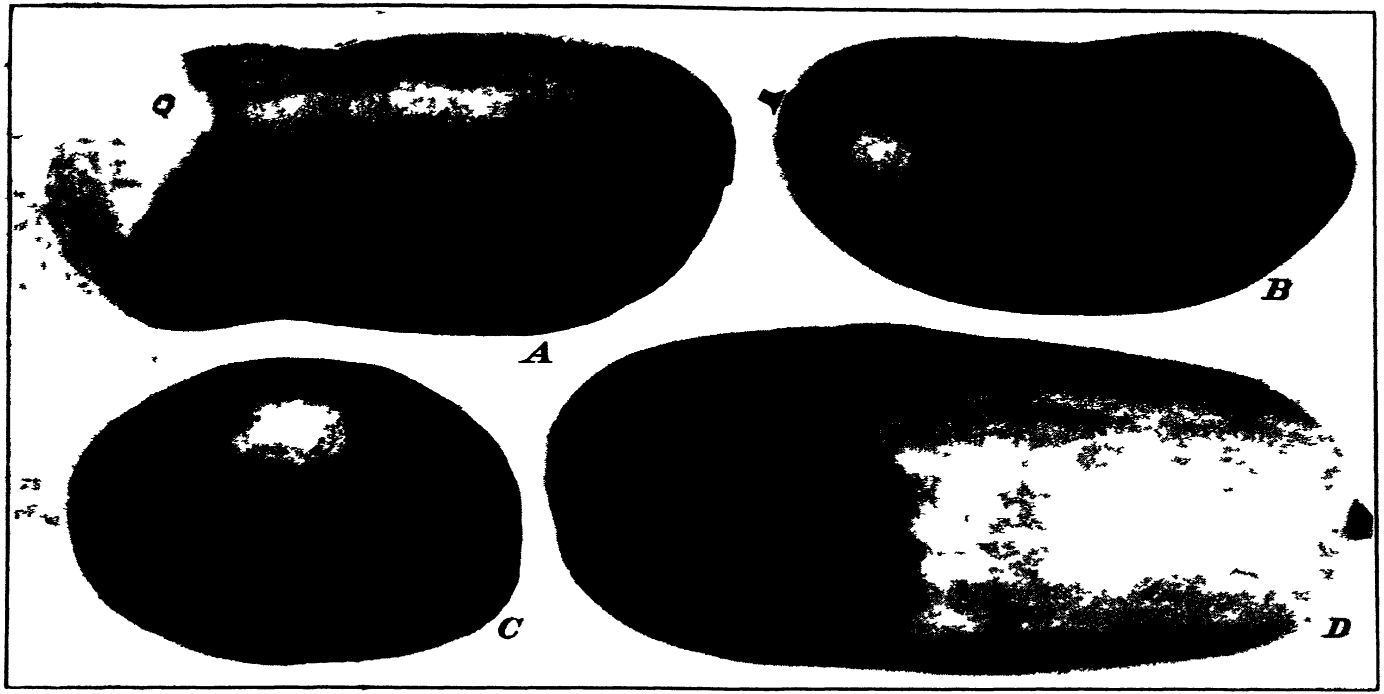

FIG 1 Watermelon fruits affected with blossom end rot due to *Pythium acanthicum*, approximately × ¼ A Specimen of the variety Irish Gray, in large part decayed as the result of spontaneous infection in the field, from the affected tissues pure cultures of the fungus were obtained. B, C Specimens of the variety Tom Watson naturally infected in the field, from both specimens pure cultures of the parasite were obtained D Specimen of the variety Irish Gray 10 days after artificial inoculation at the blossom end with a pure culture of the fungus that had been isolated from a longitudinal canker near the base of a watermelon stem.

periment Farm in September, 1922, usually extending several centimeters along the basal portions of the stems. When inoculated into sound watermelons these cultures caused dark-brown or bluish-brown decay (Fig. 1, D), indistinguishable from the decay following inoculation with cultures derived from naturally infected fruits.

In pure culture on an agar medium rich in nutrient materials, as, for example, Lima-bean-decoction agar, *Pythium acanthicum* gives rise to a submerged lustrous radiating mycelium that often displays the kind of regional variegation in density manifested to the naked eye in a cumulous appearance. On media less rich in nutrients, such as maize-meal agar, the wholly submerged mycelial growth is often so nearly indiscernible to the naked eye that the presence of a growing fungus would, on ordinary scrutiny, not be surmised. The parasite is slower with respect to rate of mycelial extension than the more widely known congeneric forms causing damping-off of seedlings; besides being easily distinguishable from the latter in its more delicate mycelial habit and its very meager emission of extramatrical hyphae.

Development of zoosporangia with production of zoospores can be induced conveniently by excising small pieces of newly invaded tissue from an affected melon, and keeping them bathed in a shallow layer of fresh water. A number of asexual reproductive units obtained through such irrigation that are shown in figure 2, A–I, and figure 3, I–K, present a rather usual range of variations in the dimensions of the 1 or more rarely 2 subspherical parts making up most of the individual sporangium, together with concomitant variations in the length and position of the hyphal part or parts making up the remainder of the sporangial body. Comparable variations are evident, too, with respect to place of origin, length and orientation of the evacuation tube (designated throughout by the letter *t*). In the assortment of reproductive units shown are represented 10 different strains of the fungus, each derived from a separate lesion. Of these 10 strains one (Fig. 2, A, a–k) was isolated from a cankered watermelon stem in 1922; another (Fig. 2, H, a, b) was isolated from a watermelon fruit with blossom-end rot, selected at Arlington Experiment Farm in 1922; 6 (Fig. 2, B, a–e; Fig. 2, D, a–e; Fig. 2, E; Fig. 2, F, a–c; Fig. 2, I, a, b; Fig. 3, G–I) came from separate watermelon fruits affected with blossom-end rot collected near Williamsburg, Maryland, in 1923; and 2 (Fig. 2, C, a–d; Fig. 2, G) came from a pair of decaying watermelons collected near Diamond Springs, Virginia, in 1923. Strain differences in morphology of sporangial apparatus, if recognizable at all, are assuredly little pronounced.

Asexual reproduction ensues very satisfactorily also when pieces of substratum permeated with well-nourished young mycelium are removed from plate cultures of Lima-bean agar and irrigated by successive changes of fresh water. On that medium, as in watermelon tissue, *Pythium acanthicum* gives rise readily to an abundance of biciliate zoospores (Fig. 3, L) that, after a period of motility, become quiescent and round up (Fig. 3, M) to germinate later by the production of germ tubes (Fig. 3, N, a–c). Though liberation

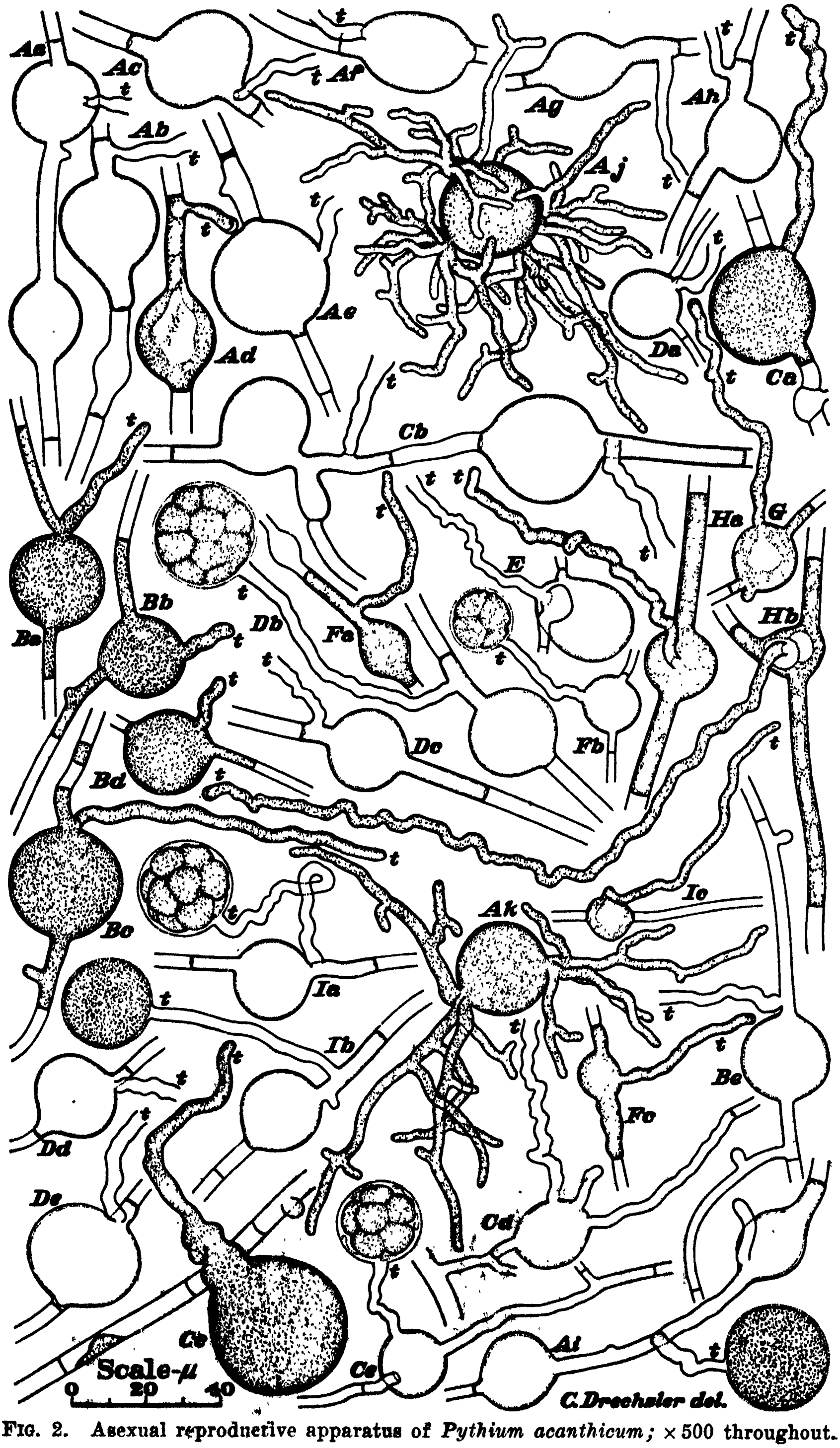

FIG. 2. Asexual reproductive apparatus of *Pythium acanthicum*; × 500 throughout.

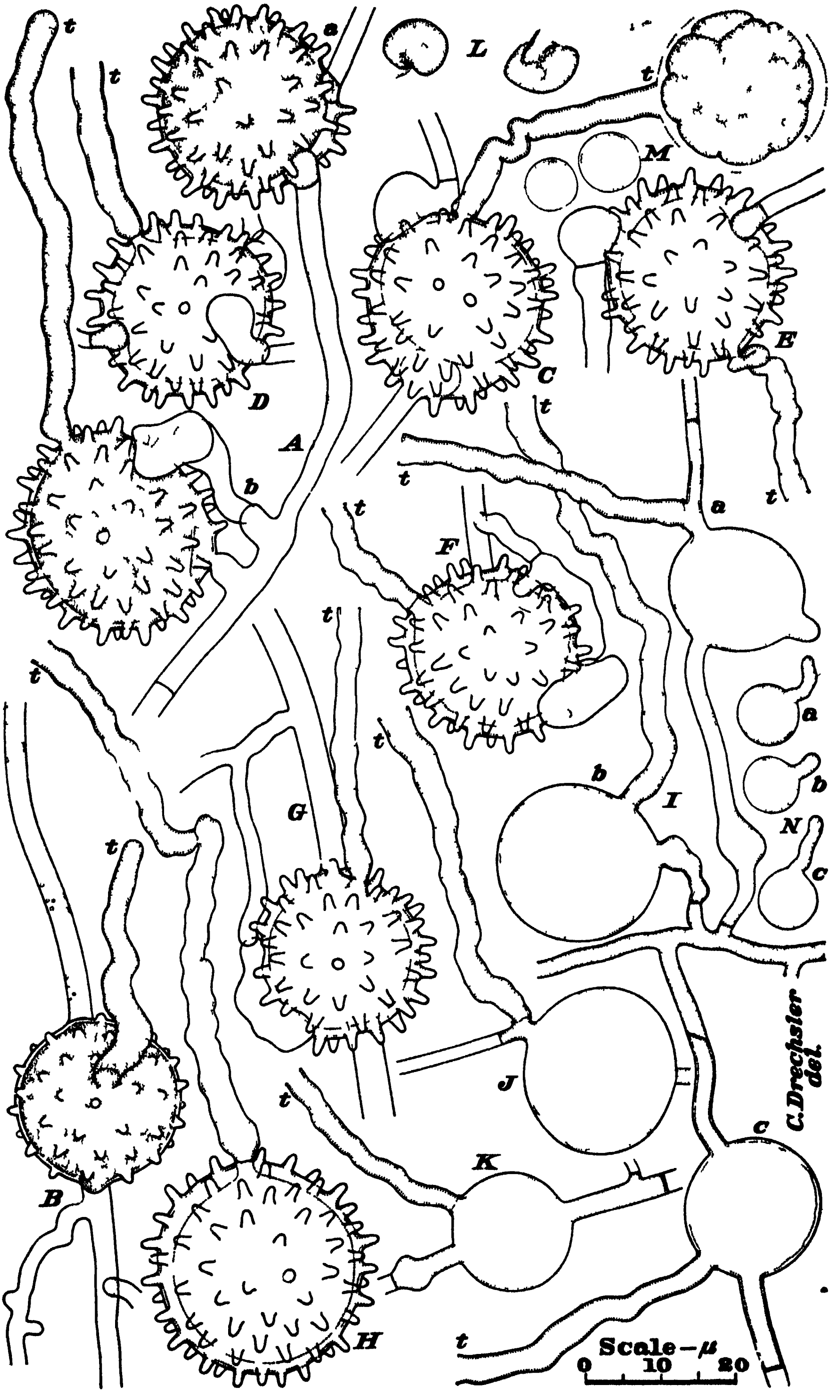

FIG 3. *Pythium acanthicum* A–H Germination of oospores by production of zoospores. I–K. Emptied asexual sporangia L–N Zoospores in successive phases. × 1000 throughout.

of zoospores may be regarded as the normal function of all asexual reproductive units that are formed in irrigated material, many globose structures, whether because of increasing bacterial contamination or because of the presence of excessive soluble food materials, germinate vegetatively by putting forth germ tubes, often in considerable numbers (Fig. 2, A, j, k).

It appears unlikely that under natural conditions *Pythium acanthicum* in watermelon fruits affected with blossom-end rot can give rise to zoosporangia. However, sexual apparatus is normally developed very abundantly in invaded watermelon tissue. The enormous numbers of mature oospores resulting from such development usually reveal the organization of contents that here, as in most oomycetes, is associated with longevity in these bodies,—the single central reserve globule being surrounded by a parietal layer of granular protoplasm wherein is imbedded a single oblate ellipsoidal refringent body. Accordingly, when their succulent matrix undergoes foul putrefaction previous to disintegration, the oospores show little degeneration and correspondingly little reduction in power of germination.

As the manipulations necessary for mounting the flaccid host tissues on glass slides usually entail some disturbance of parts, the morphology of the sexual apparatus of *Pythium acanthicum* can be studied more satisfactorily in thin slices of a moderately hard transparent gel. Maize-meal-decoction agar containing in suspension a moderate amount of fine maize-meal sediment, well distributed by agitation shortly before the medium is ready to solidify, has been utilized to advantage; its chemical composition encourages copious development of sexual apparatus with production of correctly organized oospores in immense numbers, and its physical consistency permits the cutting of paper-thin slices suitable for microscopic examination under objectives of high magnification. In figure 4, A–P, are shown 16 units of sexual apparatus drawn undisturbed from a preparation of such maize-meal agar made from a culture originally derived from a decaying watermelon fruit collected near Decker, Indiana, in 1924. The assortment of sexual units illustrates the usually intercalary position of the oogonium (Fig. 4, B–P); the usually rather even distribution of the slightly tapering, bluntly rounded oogonial protuberances (Fig. 4, A–N, P); the occasional paucity of such protuberances, especially on oogonia of small size (Fig. 4, O); the origin of the usually single (Fig. 4, A–I) antheridium from the oogonial hypha a short distance from the oogonium; the delimitation of the female organ mainly by massive plugs; the frequently, though not invariably, broad application of the antheridium; and the pluralism of reserve globules in the oospore during a protracted period of early maturity (Fig. 4, A–E, G, H) preceding their coalescense at full maturity (Fig. 4, F, I–P).

With respect to dimensions of oogonium and oospore, different strains of *Pythium acanthicum*, in comparison to one another, display a moderate range of variability, such as would seem to be usual among the more stable species of oomycetes, rather than such a wider range as was found characteristic of the graminicolous parasite *P. arrhenomanes* Drechsl. by Rands and Dopp

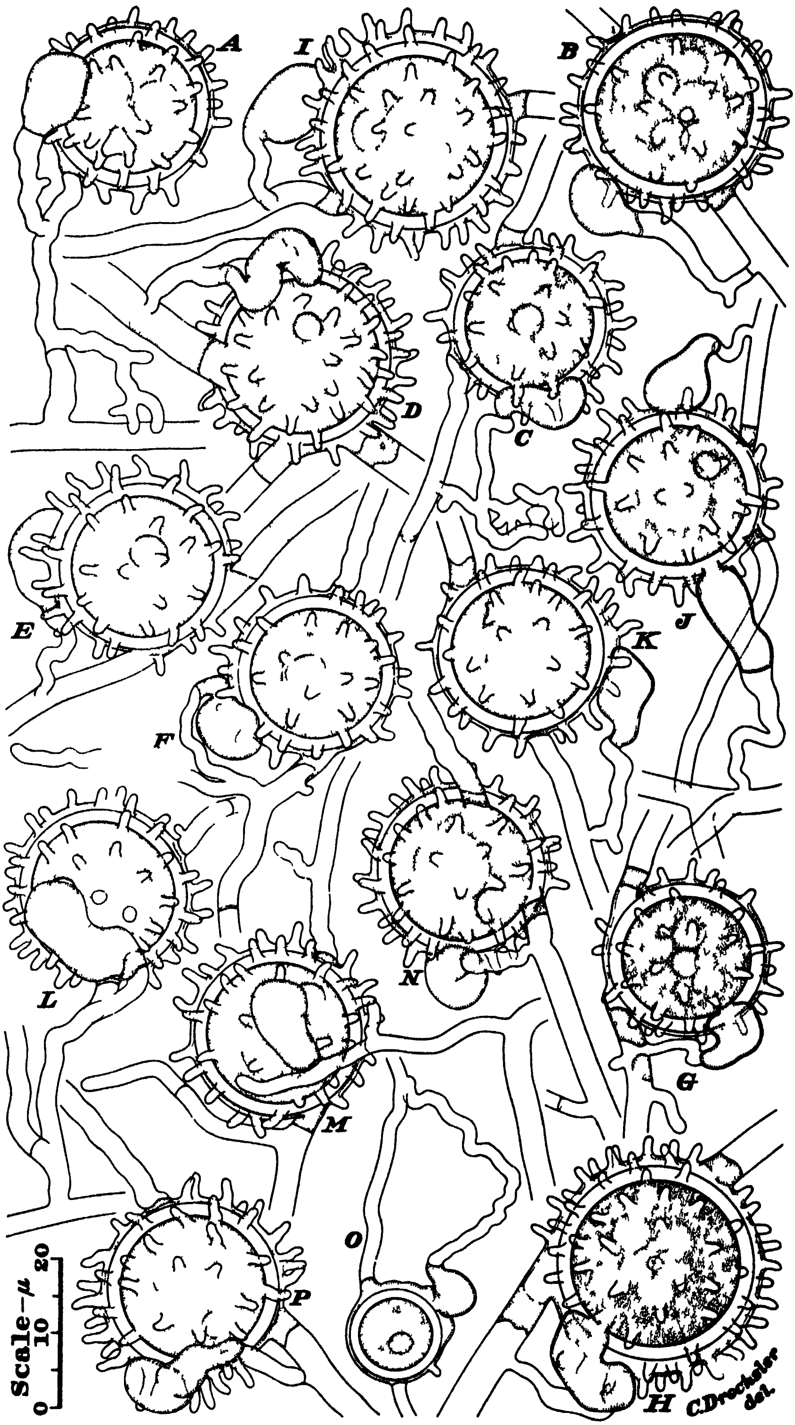

FIG 4 A–P Sexual apparatus of *Pythium acanthicum*; ×1000 throughout

(28). The relevant metrical data submitted in the diagnosis of the species were based on measurements of the same strain from Decker, Indiana, that was used in preparing the illustrations shown in figure 4. The 200 oogonia chosen at random in their maize-meal-agar matrix gave the following distribution of values for diameter expressed to the nearest micron: 13 μ, 1; 18 μ, 2; 19 μ, 4; 20 μ, 5; 21 μ, 11; 22 μ, 25; 23 μ, 40; 24 μ, 52; 25 μ, 30; 26 μ, 15; 27 μ, 7; 28 μ, 5; 29 μ, 2; 30 μ, 1. Measurements of the 200 oospores contained within these oogonia gave a distribution of values for diameter, as follows: 12 μ, 1; 15 μ, 1; 17 μ, 3; 18 μ, 5; 19 μ, 7; 20 μ, 29; 21 μ, 41; 22 μ, 49; 23 μ, 39; 24 μ, 10; 25 μ, 8; 26 μ, 5; 27 μ, 2.

Oospores of the fungus germinate freely, without any rest period, at any time after attaining approximate maturity. In pure water free of nutrient substances, germination begins with dispersion of the large reserve globule and disappearance of the thick oospore wall (Fig. 3, A, a). The protoplast expands to fill the oogonial chamber, except for the narrow protuberances (Fig. 3, A, b; B), and pushes through the oogonial wall a narrow process that, after some widening and considerable elongation, becomes recognizable as an evacuation tube (Fig. 2, A, t; B, t). With inflation of the gelatinous apex of this tube (Fig. 3, C, t) the granular contents are delivered into a terminal vesicle, there to be fashioned into zoospores in exactly the same manner as in vesicles resulting from dehiscense of asexual sporangia. The oogonium, thus emptied, now reveals in its interior a delicate subspherical membrane, which, from its continuity with the evacuation tube (Fig. 3, C–H, t) presumably represents an envelope that must have continued to surround the oosporic protoplast after the thick oospore wall had disappeared.

In fresh water containing nutrient substances and, of course, on unstaled solid artificial media, oospores of *Pythium acanthicum* germinate in the more commonplace manner, by the production of vegetative hyphae. Kept in the stale substrata in which they were developed, they retain their normal internal structure, and with it their vitality, rather longer than oospores of various other members of the genus. When protected from excessive evaporation cultures of the fungus in tubes of maize-meal agar have always been found to yield new growth promptly on being transferred to fresh substratum after 3 years of storage. The enduring vitality and ready germination of its oospores evidently serve the fungus well in meeting the peculiar difficulties that must ordinarily be associated with blossom-end infection by soil-borne parasites.

PYTHIUM PERIPLOCUM

The decay of watermelons caused by *Pythium periplocum,* whether following spontaneous infection at the floral scar (Fig. 5, A) or following artificial inoculation by incision through the stone-cell layer (Fig. 5, B), is closely similar in outward appearance to that caused by *P. acanthicum.* Externally, it is manifested by the advance of chocolate-brown, dark-brown or bluish-brown discoloration; internally it is accompanied by pronounced softening,

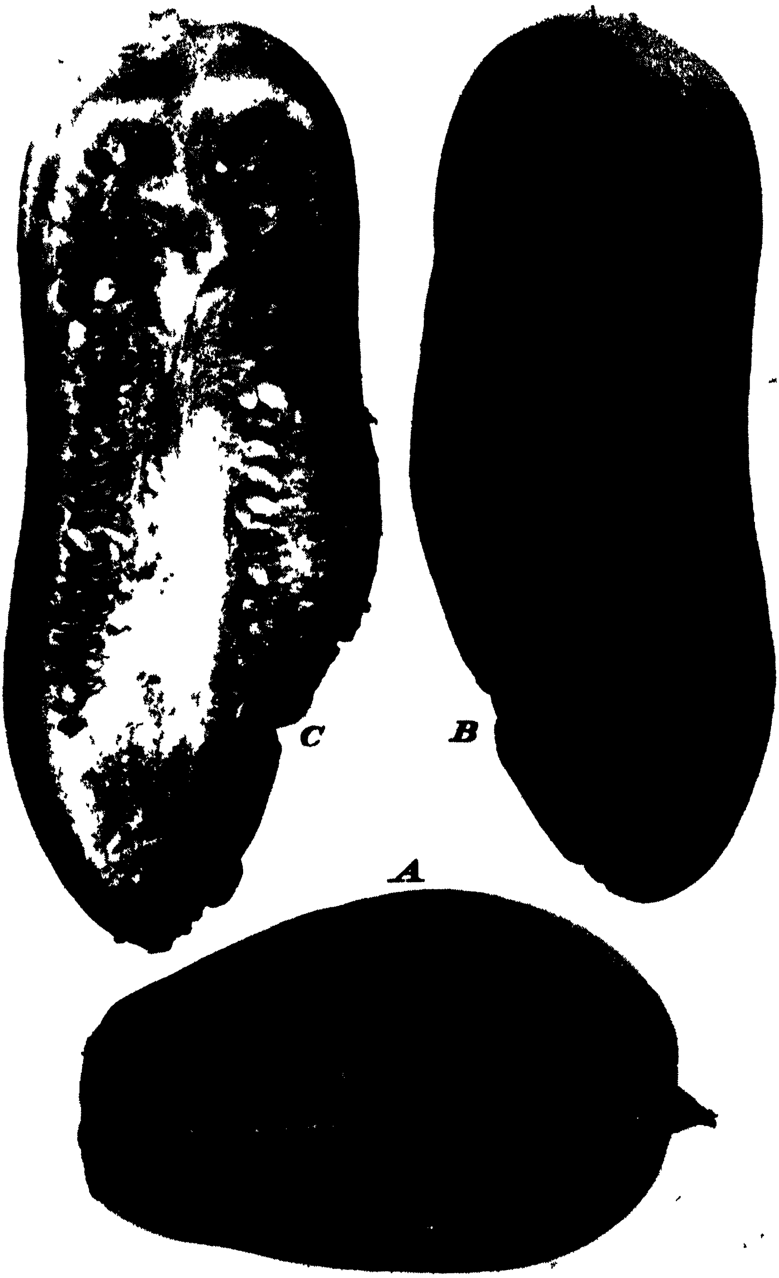

FIG. 5. Watermelon fruits affected with blossom-end rot due to *Pythium periplocum;* approximately × ¼. A. Specimen of the variety Irish Gray in process of decaying following spontaneous infection in the field; from the softened invaded tissues cultures of the parasite were readily obtained. B. Specimen of the variety Irish Gray 15 days after inoculation with a pure culture of *P. periplocum,*—the inoculation having been made by incision at the apical end of the fruit, which was kept attached to the living vine until photographed. C. Longitudinal section of the specimen shown in B.

especially of the large-celled tissues, destruction of the red pigment normal in the pulp of ripening fruit, and diffusion of a dilute sepia stain (Fig. 5, C). Yet, however strongly the 2 congeneric parasites may resemble one another in pathological effect, *P. periplocum* is encountered far less frequently than the other, and may, indeed, be regarded as a somewhat rare fungus. It was isolated from 2 watermelons affected with blossom-end rot at Arlington Experiment Farm in September, 1932; from 1 affected melon found near Williamsburg, Maryland, in August, 1923; and from 5 affected melons collected near Diamond Springs, Virginia, in August, 1923. Apart from diseased watermelon fruits, it came to light, together with *P. irregulare,* in a maize-meal-agar plate culture planted with pieces excised from cuttings of the sand pear, *Pyrus serotina* Rehd., that, on attempted propagation in March, 1923, in a nursery at Ludowici, Georgia, had failed to put forth roots.

On artificial media *Pythium periplocum* extends its mycelium with a rapidity that may be considered moderate for members of the genus. The resulting cultures present somewhat the same appearance to the naked eye as parallel cultures of *P. acanthicum,* though a difference is usually recognizable in the presence of some aerial mycelium. Under a microscope the submerged mycelium is distinguished more especially by a conspicuous development of abundantly and intricately ramifying branches attached at intervals laterally to the fairly straightforward axial hyphae (Fig. 6, A).

When well-nourished vegetative mycelium, either in newly invaded watermelon tissue, or in a rich agar medium, as, for example, Lima-bean agar, is irrigated by shallow immersion in fresh or possibly sterile water, some of the closely ramifying branches become increasingly and irregularly distended (Fig. 6, B, C) to give rise, alone or in union with similar structures, to massive lobulate zoosporangial complexes (Fig. 6, D, E). The individual complex, together often with a portion of axial hypha, simple or branched, is delimited through the deposition of a septum or of plural septa, and an evacuation tube is put forth (Fig. 6, D, t) that after attaining some length comes to bear a refringent cap at its expanded tip (Fig. 6, E, t). On receiving the discharged protoplasmic contents of the lobulate structure, this cap is inflated into a thin vesicular membrane (Fig. 6, F, t), which persists as a protective envelope during the period of 15 to 30 minutes when the granular material is being fashioned into zoospores after the manner familiar in species of *Pythium* (Fig. 6, G, t). While a single evacuation tube (Fig. 6, H, t) ordinarily suffices for the dehiscense of a sporangial complex of moderate volume, 2 or even 3 evacuation tubes (Fig. 6, K, t, t, t) often serve in the discharge of unusually massive complexes. Rupture of a vesicle often liberates from 100 to 125 actively motile zoospores that, except for occasional instances of incomplete cleavage (Fig. 6, J, b), consist of a somewhat reniform protoplast bearing 2 lateral cilia (Fig. 6, J, a). The organs of locomotion are made more distinctly visible in preparations exposed to osmic acid fumes and stained with gentian violet (Fig. 6, K). After a variable period

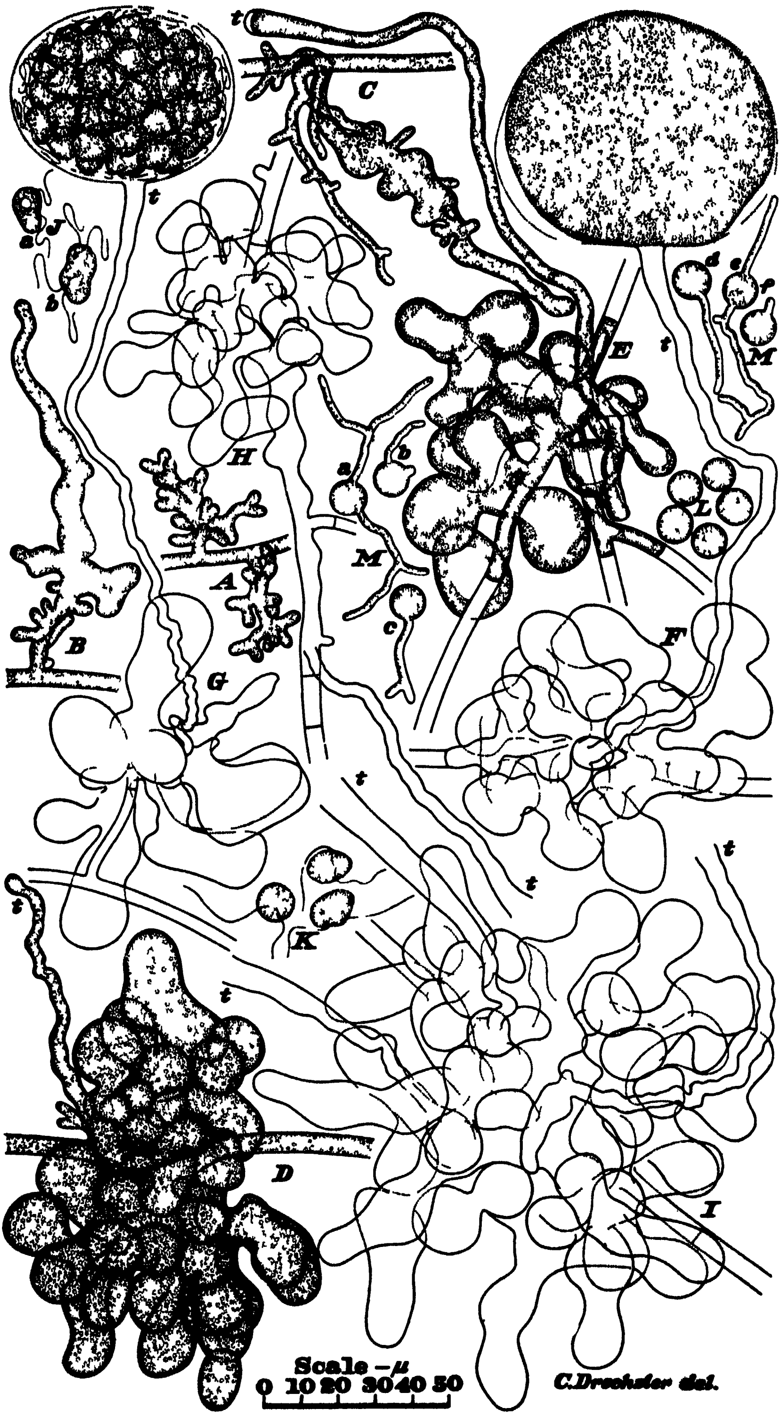

FIG 6. Mycelium and asexual reproductive apparatus of *Pythium periplocum*; × 500.

of motility the zoospores round up (Fig. 6, L) to germinate later by the production of 1 or 2 germ tubes (Fig. 6, M, a–f).

In its nutritional requirements for sexual reproduction, *Pythium periplocum* is more exacting than the more widely known damping-off parasites congeneric with it, inviting comparison rather with such refractory forms as *P. arrhenomanes* and *P. scleroteichum* Drechsl. (19). On many media of wide usefulness, as, for example, potato-dextrose agar, the mycelium may give rise abundantly to oogonia and antheridia, but the sexual organs promptly degenerate, often without producing any good oospores whatever. Normal development of oospores is better encouraged through use of maize-meal-decoction agar containing in suspension a considerable quantity of the finer maize-meal sediment. When paper-thin slices, cut with a razor from the plane surface of a culture prepared with this medium, are mounted under thin cover glasses and examined microscopically under an objective of high magnification, the rather intricate arrangement of the sexual apparatus may be made out satisfactorily.

The intricacy of the individual unit of sexual apparatus is attributable mainly to the antheridia, which, in numbers varying usually from 1 to 4, are borne on a single branch (Fig. 7, A, C, D, F, G, H, J, K) without close mycelial connection with the oogonium, or, more rarely, on 2 such branches (Fig. 7, B, E). As each antheridium consists typically of a longish structure bearing a number of ventrally protruding lobes, and, in addition, may in various ways be branched more or less, the oogonium is often surrounded by its male complement in a manner recalling certain species of the saprolegniaceous genera *Aphanomyces* and *Plectospira* (15, 16).

While in *Pythium periplocum* the female organ would seem to be formed terminally somewhat more often than in *P. acanthicum,* the difference in positional relationship cannot be considered a very pronounced one. Nor are wide departures from the morphology of the latter species evident in the spiny ornamentation of the oogonium, or in its dimensions. Measurements of the 200 oogonia selected at random, from which were derived the relevant metric data submitted in the diagnosis, showed a distribution of values for diameter expressed to the nearest micron, as follows: 15 μ, 1; 16 μ, 1; 21 μ, 5; 22 μ, 17; 23 μ, 25; 24 μ, 38; 25 μ, 50; 26 μ, 39; 27 μ, 11; 28 μ, 8; 29 μ, 2; 30 μ, 2; 32 μ, 1. Measurements of the oospores contained within these oogonia showed a distribution of values for diameter, as follows: 13 μ, 1; 14 μ, 1; 16 μ, 1; 17 μ, 2; 18 μ, 7; 19 μ, 14; 20 μ, 39; 21 μ, 48; 22 μ, 48; 23 μ, 24; 24 μ, 13; 26 μ, 1; 27 μ, 1. Comparison of the averages computed from the 2 sets of measurements with the corresponding averages computed from the homologous measurements of *P. acanthicum* indicates a somewhat greater oogonial diameter and a slightly smaller oospore diameter in the present species. Because of this dimensional proportionality the oogonial cavity here is noticeably less nearly completely filled by the oospore, which, in its fully mature state, reveals the internal organization familiar in numerous oomycetes,—a single large central reserve globule of homogeneous consistency being sur-

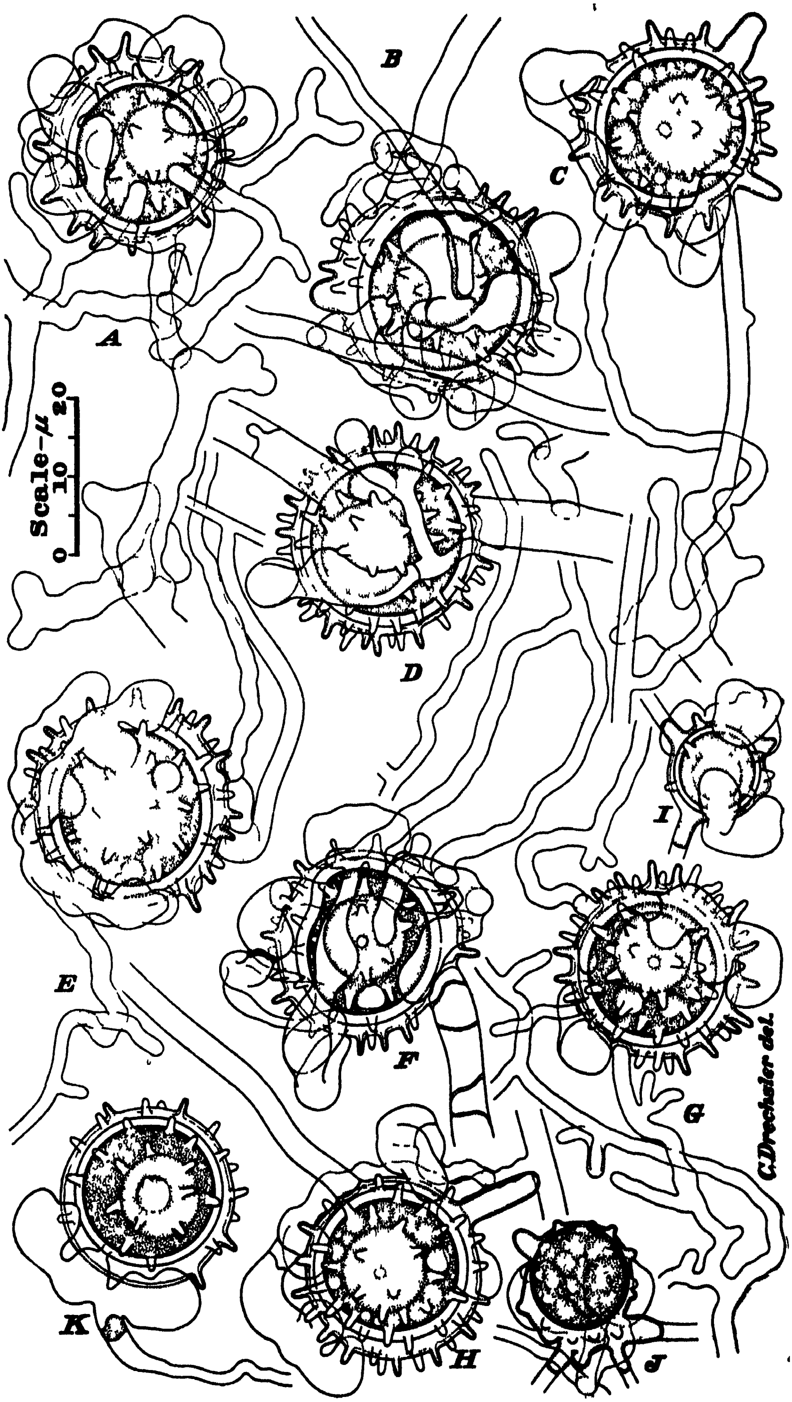

FIG. 7. A–K. Sexual apparatus of *Pythium periploocum;* ×1000 throughout.

rounded by a parietal layer of granular protoplasm in which is imbedded a single subspherical or oblate ellipsoidal refringent body (Fig. 7, A–I, K).

PYTHIUM HELICOIDES

Pythium helicoides has been identified in only one lot of cultures isolated by me from watermelons affected with blossom-end rot. Of the 48 cultures in that lot, all of which were obtained from separate decaying specimens chosen at random on August 18, 1937, in an experimental field belonging to the U. S. Regional Vegetable Breeding Laboratory at Charleston, South Carolina, 7 are referable to the fungus in question. The very wet conditions that had prevailed in the field during the week before the material was collected may very probably have facilitated the unusual parasitism of the organism in a plant structure neither subterranean nor aquatic. It may be presumed that as with other fungi causing blossom-end decay the initial difficulty in gaining a foothold within the uninjured fruit provides the chief protection of the host; for, when *P. helicoides* in pure culture is introduced into watermelons by incisions through the stone-cell layer, decay manifested externally in dark-brown discoloration (Fig. 8, A, B) follows with no less certainty than after similar inoculation with *P. acanthicum.*

In pure culture on artificial media the fungus extends its mycelium nearly as rapidly as the species of *Pythium* causing damping-off. A general similarity to such forms as *P. ultimum* and *P. irregulare* is evident, too, in the macroscopic appearance of its growth on agar media; though the resemblance is often largely lost after cultures attain an age of several weeks, when the aerial mycelium of the common seed-bed parasites will ordinarily, even in the absence of contaminating bacteria, have collapsed to the surface of the substratum as a moist fibrous mat, whereas the aerial mycelium of *P. helicoides* will usually persist for years in its cottony state. This persistence of aerial mycelium, long familiar in various species of *Phytophthora,* is conspicuous also in cultures of the 3 fungi I have described (17) under the names *Pythium oedochilum, P. polytylum* and *P. palingenes,*—fungi that, from their morphological parallelism with *P. helicoides,* must be regarded as closely related to it.

Unlike most of the species of *Pythium* familiar to students of plant diseases, *P. helicoides* does not generally give rise either to zoosporangia or to their morphological equivalents, conidia, under conditions wholly unfavorable for immediate production of zoospores. A dry substratum usually encourages little or no development of asexual reproductive apparatus. However, when sizable tracts of well nourished vegetative mycelium in pieces of maize-meal agar or Lima-bean agar are transferred to a shallow layer of fresh water, they extend into the liquid new filaments of considerable length that often give off distally in racemose or cymoid arrangement a number of shorter branches, some of which may in turn give rise to secondary branches. On the axial filament as also on the individual ramification is borne terminally a subpherical or obovoid zoosporangium (Fig. 9, A, a–h) that, mostly from its

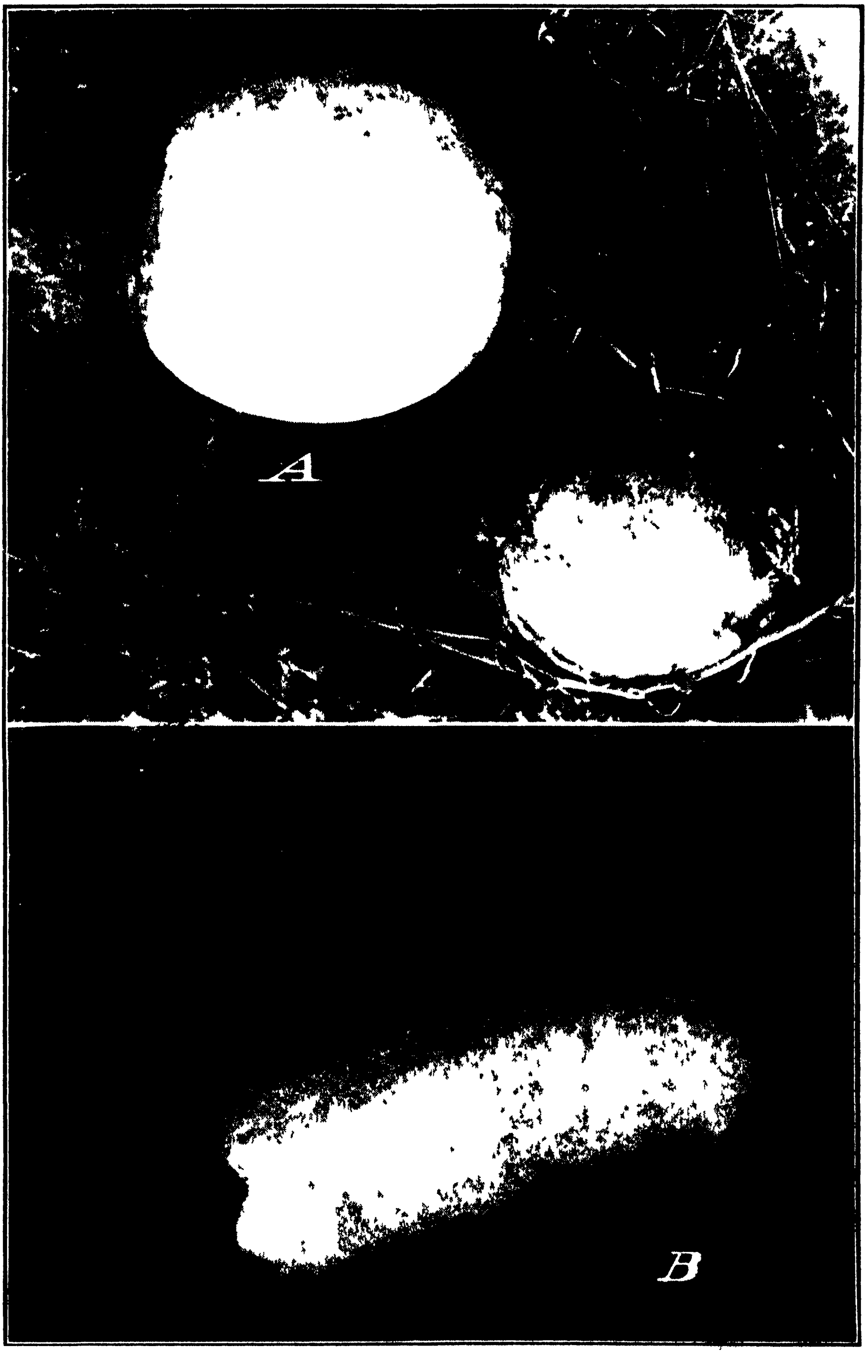

FIG. 8. Watermelon fruits 10 days after inoculation with a pure culture of *Pythium helicoides*,—the inoculation having been made in each case through an incision at the apical end of the fruit, which was kept attached to the living vine until photographed; approximately × ⅛. A. Specimen of the variety Northern Sweet. B. Specimen of the variety Burrell's Gray.

apex or from a part close thereto, thrusts out a sturdy and often rather short evacuation tube. When the undifferentiated sporangial contents are discharged through this tube they accumulate in a vesicle formed by inflation of its refractive gelatinous cap and are then fashioned into biciliate reniform zoospores after the manner typical of the genus (Fig. 9, B, L, I, M, O, Q). External conditions making for functional frustration of an evacuation tube may be reflected in somewhat unusual elongation of this structure (Fig. 9, N), or in production of one or more supernumerary tubes (Fig. 9, R, S, T, U). Following a variable period of motility the zoospores come to rest and round up (Fig. 9, X) either to produce secondary swarmspores somewhat later by repetitional development (Fig, 9, Y, a, b), or more usually to germinate vegetatively (Fig. 9, Z).

In common with many other of the aquatic or semiaquatic Pythiaceae that bear globose, ovoid or obovoid sporangia terminally on more or less submerse filaments, *Pythium helicoides* shows a proliferous tendency in its asexual reproduction (Fig. 9, A, g; H). On discharge of a sporangium the supporting hypha often gives rise to a second sporangium, which may be borne sessile within the empty envelope of the first (Fig. 9, C, J, P, V, W), or may be borne terminally on a hyphal prolongation passing lengthwise through the empty envelope and its empty evacuation tube (Fig. 9, D). By repetition of the process a third sporangium may occasionally be observed developing within the evacuated membrane of the second (Fig. 9, E). Somewhat similar multiplication comes about when from a position immediately below one sporangium, whether discharged or not, the supporting filament grows out laterally to produce another, now directly on the axial hypha, now terminally on a lateral prolongation (Fig. 9, F, G, K, L, T, U).

In all essential details of morphology and development the asexual reproductive phase of *Pythium helicoides* reveals unmistakable parallelism with the zoosporangial stage which de Bary (2) described very well in 1860, and on which, in the absence of a sexual stage, he then established his *P. proliferum*. Later, fortunately, that species was given a more precise definition in a revised characterization (3, 4) based on a fungus producing in conjunction with proliferous sporangia sex organs very closely resembling those set forth by him as pertaining to *P. debaryanum;* the usually intercalary oogonium being fertilized in most instances by 2 or 3 antheridia consisting predominantly either of adjacent portions of hypha, or of short lateral branches arising from the oogonial hypha close to the female organ. The ripe oospore of *P. proliferum* was stated by de Bary (4, p. 559) to be provided with a more prominent and more highly refractive central reserve globule than that of *P. debaryanum.* The figure (4, fig. 19) given by him of this structure shows a single central reserve globule that would seem, indeed, of relatively large size; and, what is of no less importance, depicts unmistakably a single refringent body ("heller Fleck") lying in the narrow parietal layer.

There is reason to presume that in the main the application of *Pythium proliferum* has been governed more by general agreement with the type of

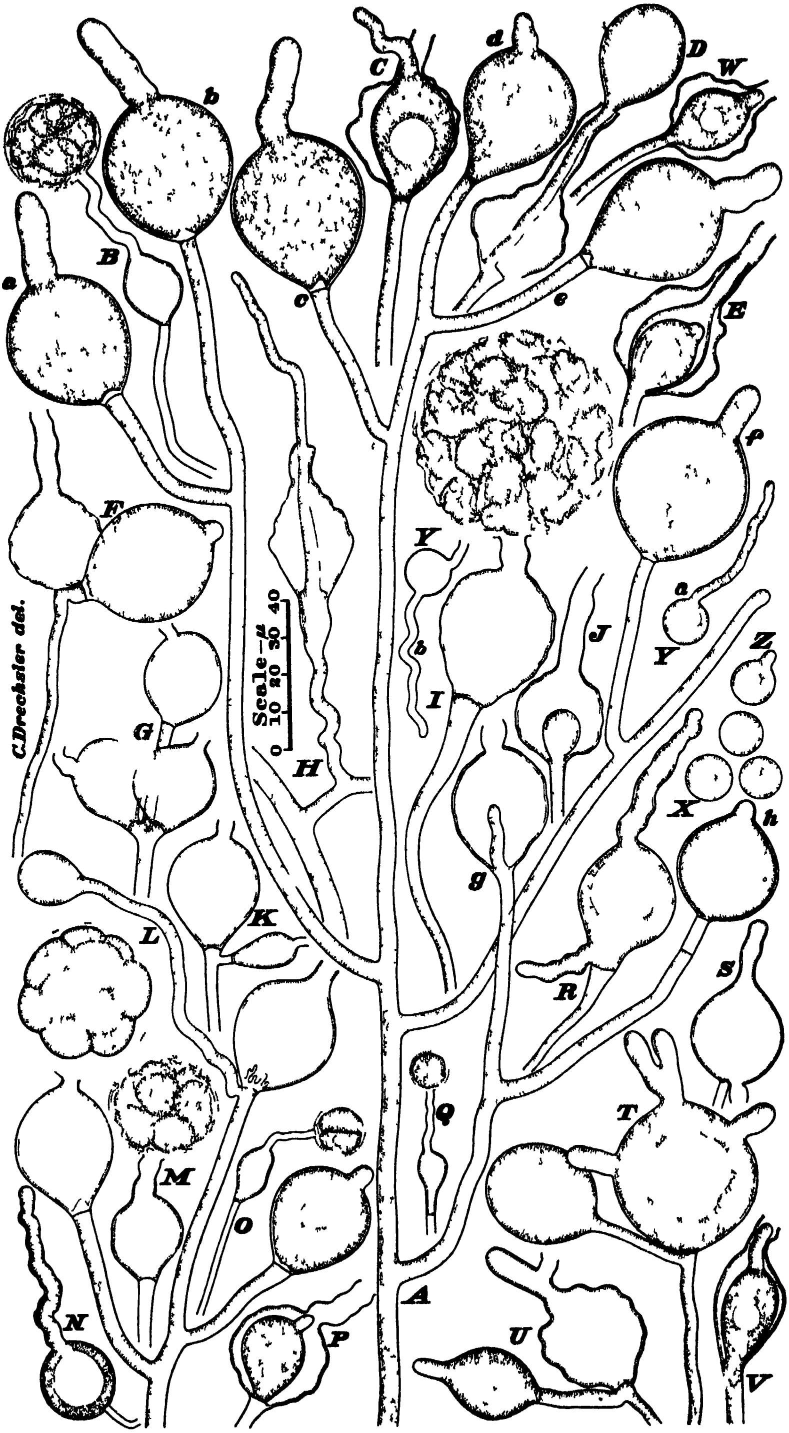

FIG. 9. Asexual reproductive apparatus of *Pythium helicoides*; × 500 throughout.

asexual reproduction originally made diagnostic for the species than by conformity to the type of sexual apparatus later brought into the specific characterization. Citations of the binomial without descriptive detail in publications on the microflora of soil or of water, such as those of Harvey (21), Raper (28), Valkanov (29), and Lund (23), were more probably based on observed instances of zoosporangial proliferation than on recognition of correspondence with respect to morphology of oogonium, antheridium, and oospore. In Wardlaw's report (32) on the occurrence of the species on roots of the strawberry in Scotland, reference is made to mycelium, to sporangia, to zoospores, and to the process of proliferation, but no mention is made of oospores. Crooks (10), after discussing mycelium and proliferous sporangia in her recent account of *Pythium proliferum,* states that no sexual organs were observed.

Yet, some usage attributing to *Pythium proliferum* sexual apparatus, unquestionably of the same general type as that set forth in de Bary's final characterization of the species, is supplied in a number of descriptive publications by later writers. Ward (31), who obtained an abundance of oogonia in material he held referable to *P. proliferum,* declared their development and fertilization similar to these processes in *P. debaryanum,*—an assertion supplemented by several figures of which 2 show stages in the fertilization of terminal oogonia by antheridia borne terminally on branches (the branch in one instance arising from the oogonial hypha at some distance from the female organ), while a third shows oospores in each of which a single large reserve globule is surrounded by a parietal layer. Butler (7) found in his material of *P. proliferum* more antheridia arising from a neighboring hypha than were supplied by the oogonial filament, and described these organs as generally being short and little curved. Matthews' (24) illustrations of the species show antheridia that, in shape and mycelial relationships, as also in their frequently rather narrow apical contact with the oogonium, are reminiscent of the antheridia formed by *P. debaryanum* and *P. ultimum;* and in the oogonia fertilized by these decidedly commonplace male elements were formed equally commonplace oospores, which were stated to contain at maturity a central reserve globule surrounded by a granular layer of protoplasm having imbedded in it a small refractive body.

Conspicuous morphological departures from such very familiar type of sexual apparatus come to light in *Pythium helicoides.* The oogonium of this species, to be sure, offers little peculiarity, consisting merely of a subspherical enlargement densely filled with protoplasm and borne for the most part either laterally on an axial hypha (Fig. 10, A–D, F) or terminally on a lateral branch that frequently is very short (Fig. 10, G) but sometimes attains moderate length (Fig. 10, E). Much greater distinctiveness attaches to the elongated cylindrical antheridia that in numbers from 1 to 4 apply themselves very tightly lengthwise to the oogonium, becoming fused virtually from basal septum to rounded apex with the oogonial wall along an arc equivalent often to more than a fourth of the oogonial circumference. The frequently

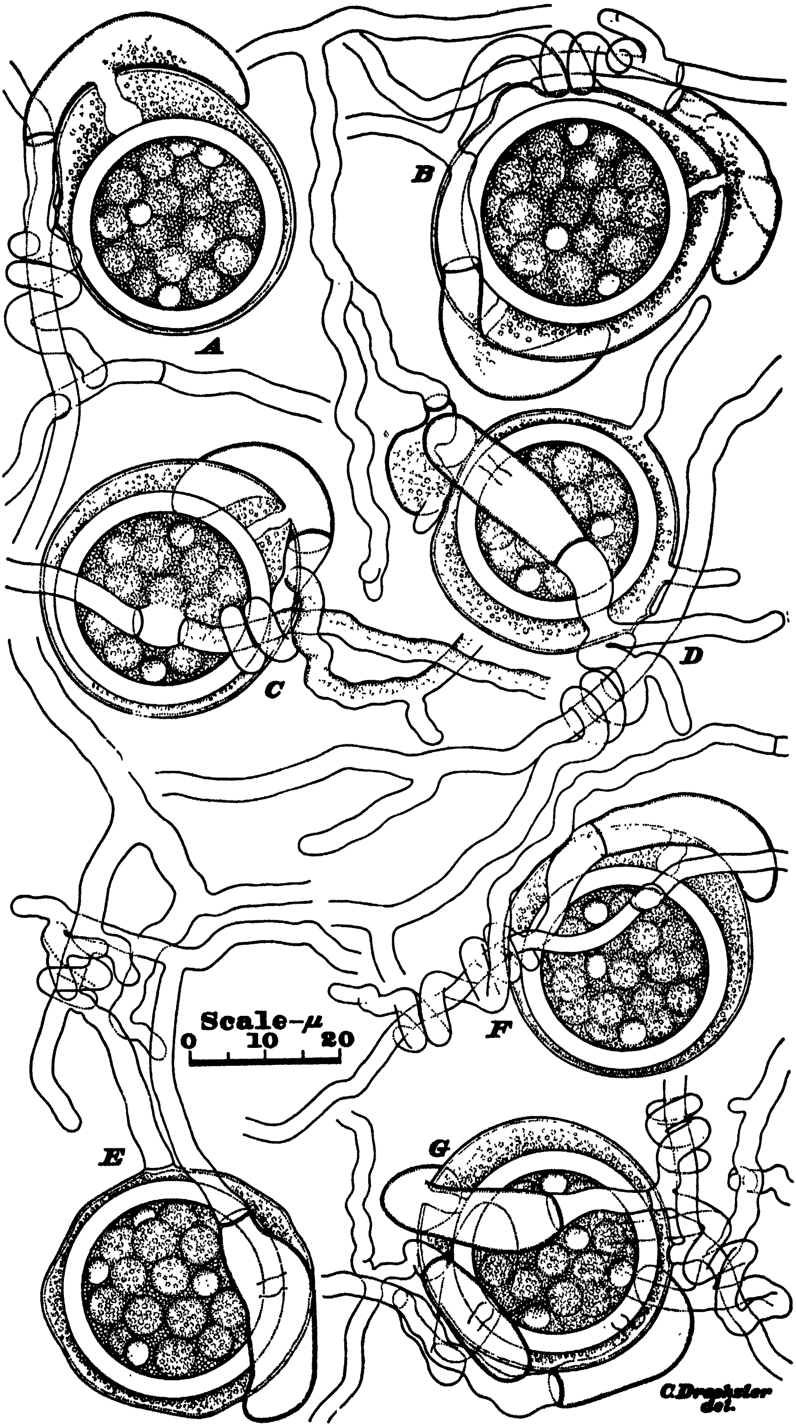

FIG. 10. A–G, Sexual apparatus of *Pythium helicoides*; × 1000 throughout.

somewhat stout fertilization tube, through which the antheridial contents are transferred, arises ordinarily from a position approximately median between the base and the apex of the male organ, rather than from an apical position, as in most oomycetes. It usually attains a greater length than the homologous structures in most species of *Pythium*,—a detail of development in many instances related to a protrusion of the oogonium at the region of contact. A very characteristic relationship of the filaments supporting the sex organs is consistently present in a helicoid involvement of an oogonial hyphal element by an antheridial hyphal element. Two close turns, comparatively regular in a geometrical sense, are usually described in each spiral, though, by juxtaposition of 2 spirals, 4 turns sometimes appear as if making up a single series (Fig. 10, F, G). At least one spiral arrangement is found associated with each unit of sexual apparatus. In units where the oogonium is fertilized by plural antheridia of separate origins, only one antheridial system may participate in the involvement (Fig. 10, D, G); or, again, additional involvement may be achieved by one or another of the supernumerary male hyphae.

The mature oospore of *Pythium helicoides*, as has been pointed out earlier (17, 20), shows a very characteristic internal organization. Its content of oily reserve material, instead of being concentrated in a single large central globule, is divided into a half-dozen to a score of smaller globules distributed more or less evenly throughout the murkily granular protoplasm. Likewise, instead of a single refringent body, 2, 3, or 4 such bodies may be discerned imbedded in the granular material at some distance from one another (Fig. 10, A–G).

Sexual apparatus of the type represented in *Pythium helicoides* was set forth also in the original descriptions of the 3 closely allied species, *P. oedochilum, P. polytylum* and *P. palingenes*. Similar apparatus has been recognized, too, in many cultures more recently isolated from various sorts of decaying plant materials. A fungus repeatedly obtained from watersoaked portions of leaves of the white water-lilies *Nymphaea odorata* Ait. and *N. tuberosa* Paine, collected in Massachusetts, New York, and Wisconsin, shows a general parallelism with *P. helicoides* not only in its oogonia, antheridia, and oospores, but in its proliferous zoosporangia, as well; and must accordingly be considered a member of the same intimate group. Whether the fungus in question is identical with the one isolated by Dissmann (12) from leaves of *N. candida* Presl. in Central Europe and discussed by him at length under the binomial *P. proliferum*, remains problematical. The elongated clasping antheridia described and figured as pertaining to the parasite on the European water-lily, certainly show much more similarity to the male organs of *P. helicoides* and its allies than to those ascribed to *P. proliferum* by de Bary. The "sickle-shaped bodies" that Dissmann was led by resemblances in outward shape to interpret as antheridia formed independently of oogonia, appear more correctly interpretable as appressoria. The production of such bodies at the surface of hard objects, observable in cultures of numerous species of *Pythium*, and more particularly the very copious development of homologous modifications on aerial hyphae of *P. butleri* and *P. myriotylum*, whereby unmistakably these frequently aerial parasites are enabled to force their way through unbroken epicarp of cucumber fruits, for example, seem little expressive of a sexual function

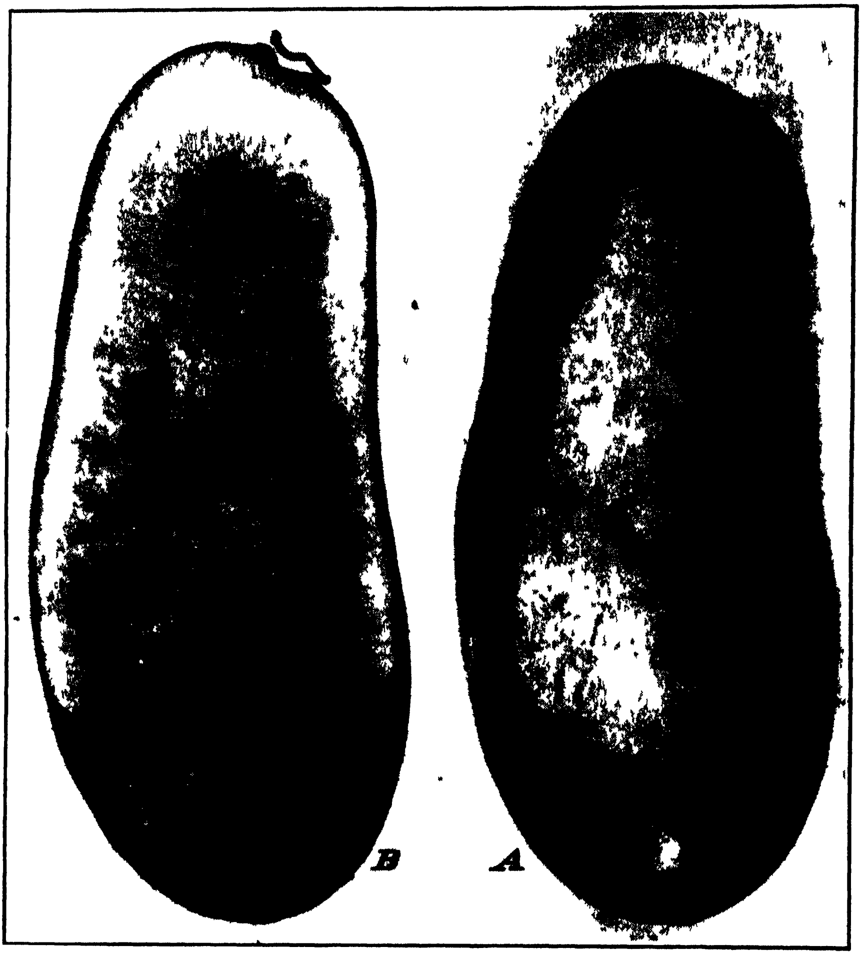

FIG. 11. Watermelon of the variety Irish Gray 8 days after inoculation with a pure culture of *Pythium anandrum*,—the inoculation having been effected by placing a bit of the culture into an incision through the stone cell layer in the region of the flower scar, in order that the invasion might simulate the development following spontaneous infection; approximately × ⅙. A. External view. B Longitudinal section along the axis of the fruit.

PYTHIUM ANANDRUM

A sexual stage reminiscent of *Pythium acanthicum* and *P. periplocum* is found combined with an asexual reproductive phase suggestive of *P. helicoides* in a fungus that I described (17) under the name *P. anandrum* in 1930, 6 years after isolating it from a decaying underground bud of a failing crown of rhubarb, *Rheum rhaponticum* L. As similarly affected crowns have not since been received for examination, opportunity for determining to what extent the fungus occurs on rhubarb buds has been lacking. It has not been recognized in some thousands of cultures derived from decaying portions of numerous other flowering plants, and would seem, therefore, to represent a comparatively rare species. On artificial inoculation into watermelon fruits it causes a fairly rapid decay that, like the blossom-end rots caused by the 3 congeneric parasites already discussed, is manifested externally by dark-brown discoloration (Fig. 11, A), and internally by dilute sepia discoloration as well as by watery softening of the tissues (Fig. 11, B).

In pure culture on maize-meal agar *Pythium anandrum* reveals a handsome mycelial habit somewhat similar to that of *P. debaryanum, P. irregulare,* or *P. mammillatum:* the axial hyphae pursuing gracefully straightforward courses, and giving off at intervals perceptibly narrower, irregularly disposed branches that in turn bear stubby ramifications and diverticula (Fig. 12). After a few days oogonia begin to develop as globose bodies borne terminally on branches of variable lengths. At first smooth (Fig. 13, A) they soon become beautifully beset with numerous tapering spiny protuberances. Maturation brings about development of a thick-walled spherical spore showing the internal organization usual for oospores,—a single large central reserve globule being surrounded by a parietal granular layer, within which is imbedded a single subspherical refringent body (Fig. 13, B–F). Though the septum delimiting the oogonium is often convexly arched toward the spore, it is not evident that a stalk antheridium is present. Since branch antheridia are assuredly always absent, development here would seem consistently parthenogenetic. Just as in many other species of *Pythium,* occasional departures from the usual are to be seen, as, for example, production of 2 parthenospores in a biloculate oogonium (Fig. 13, G), and failure of abnormally small oogonia to put forth spiny protuberances (Fig. 13, H). Degeneration is usually not serious, even when maize-meal agar is employed that contains little maize-meal sediment in suspension; the healthy character of the development being reflected in moderate uniformity of dimensions. The measurements of 200 oogonia, selected at random, from which were derived the relevant metric data submitted in the original diagnosis, showed the following distribution of values for diameter (exclusive of protuberances) expressed to the nearest micron: 23 μ, 1; 24 μ, 5; 25 μ, 7; 26 μ, 23; 27 μ, 26; 28 μ, 47; 29 μ, 36; 30 μ, 26; 31 μ, 22; 32 μ, 6; 33 μ, 1. Measurements of the 200 oospores contained within these oogonia showed a distribution of values for diameter, as follows: 19 μ, 1; 20 μ, 1; 21 μ, 11; 22 μ, 18; 23 μ, 27; 24 μ, 47; 25 μ, 31; 26 μ, 30; 27 μ, 27; 28 μ, 7.

Pythium anandrum appears rather reluctant to reproduce asexually. Yet, now and then, following transfer of young mycelium in pieces of Lima-bean agar to a shallow layer of water, it has been observed to put forth long delicate submerged hyphae bearing solitary, terminal, prolate ellipsoidal, or somewhat ovoid-sporangia, provided individually with an apical papilla of homogeneous consistency (Fig. 14, A–B, D–H). In shape, as well as in papillate condition,

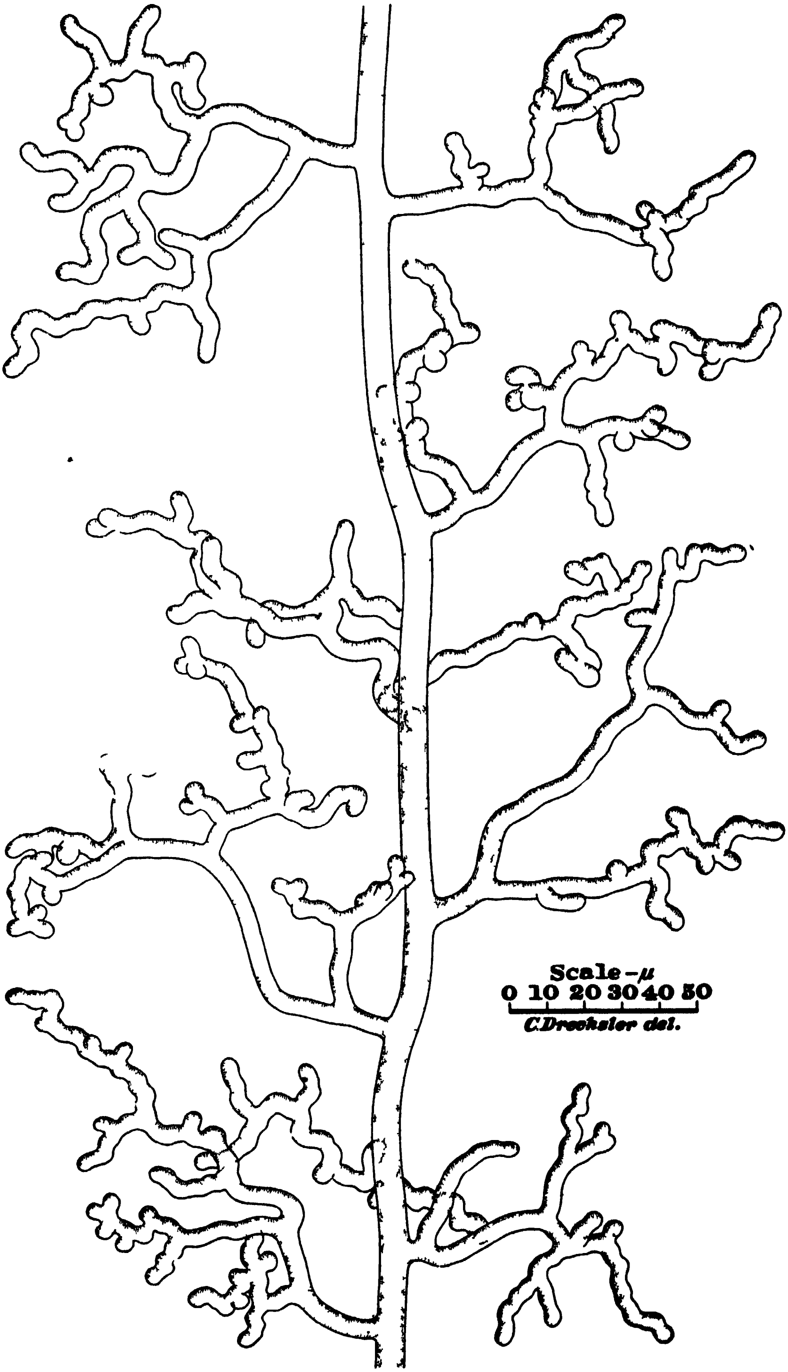

FIG. 12. A portion of submerged mycelium of *Pythium anandrum*, showing disposition of lateral branches on an axial hypha, and their manner of ramification; drawn from a maize meal agar plate culture about 5 mm back from the advancing margin of the growth; × 500.

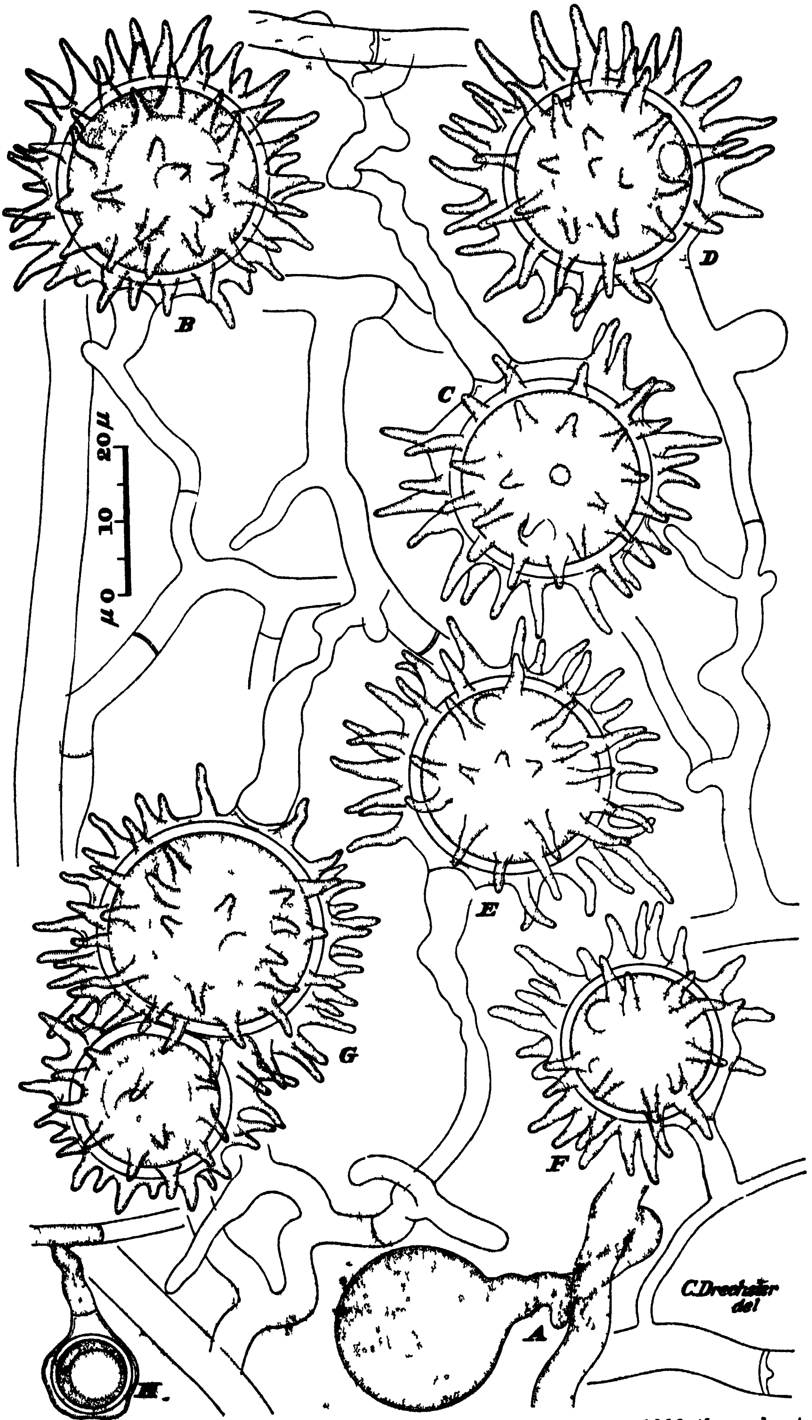

FIG. 13 A-H Sexual apparatus of *Pythium anandrum*, × 1000 throughout.

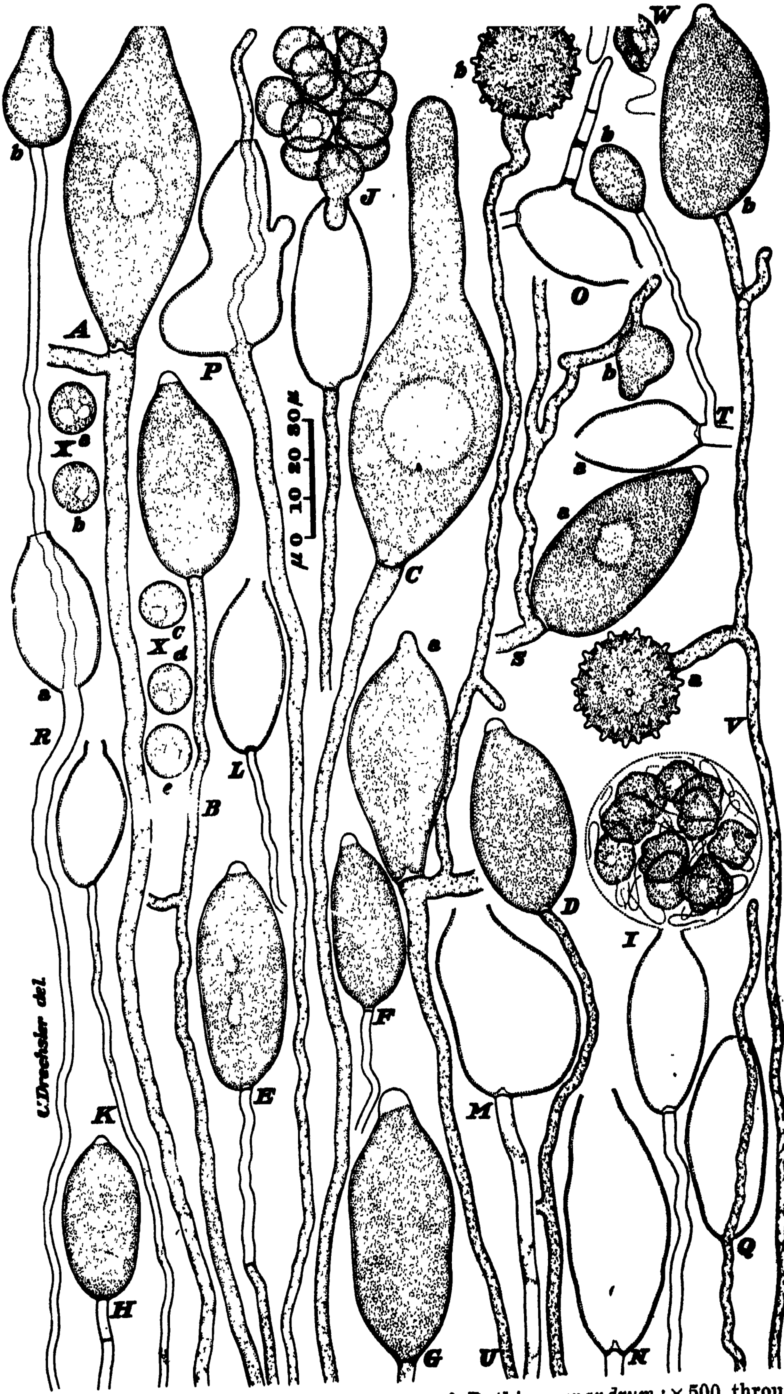

FIG. 14. Asexual reproductive apparatus of *Pythium anandrum*; × 500 throughout.

the sporangia seem comparable to those of certain species of *Phytophthora,* more particularly, perhaps, of *Phytophthora citrophthora* (Sm. & Sm.) Leonian and *P. colocasiae* Rac. Certainly, in most instances, no recognizable evacuation tube is formed preliminary to dehiscence, the sporangial contents, apparently while in a wholly undifferentiated state, being discharged directly into a sessile vesicle resulting from inflation of the papilla. Within this vesicle the relatively large biciliate zoospores are fashioned wholly after the manner usual in the genus *Pythium* (Fig. 14, I). Following rupture of the gelatinous membrane these ordinarily swim about for some time (Fig. 14, W), eventually to encyst (Fig. 14, X, a–e) in scattered positions; though, occasionally, for lack of water in sufficient quantity, they may round up in a cluster at the mouth of the sporangium (Fig. 14, J). Sometimes no further development takes place (Fig. 14, K–O), but at other times the sporangiophore resumes growth by extending itself through the empty sporangial chamber (Fig. 14, P; Q; R, a) and then bears a second sporangium farther on (Fig. 14, R, b). Often, too, a second sporangium (Fig. 14, S, b; T, b) is formed on a lateral prolongation of the sporangiophore arising from a position immediately below the basal septum delimiting the first (Fig. 14, S, a; T, a). A fertile hypha not infrequently gives rise at some little distance from the sporangium (Fig. 14, U, a; V, b) borne by it, to an oogonium (Fig. 14, U, b; V, a), which here, owing to the aquatic environment, is usually ornamented only with rather small protuberances. Because of unfavorable conditions a sporangium may suffer functional frustration, and thereupon put forth a stout process often somewhat resembling the evacuation tubes of other species (Fig. 14, C).

A combination of proliferous sporangia with spiny oogonia in one and the same fungus is recorded in de Bary's account (3, 4) of his *Pythium megalacanthum.* Since, in that account, branch antheridia from 1 to 4 in number are set forth as supplying the terminal or intercalary oogonia, sporangia are described as regularly producing evacuation tubes previous to dehiscence, and extraordinarily large zoospores are stated to round up into cysts averaging 18 to 20 µ in diameter, the species discussed is manifestly not identical with *P. anandrum.* Yet, despite the very obvious specific differences, it cannot be considered impossible that de Bary's fungus may have been a form morphologically and taxonomically less alien to *P. anandrum* than is the impressive spiny parasite from flax, *Linum usitatissimum* L., roots dealt with by Buisman (6) and by Diddens (11) under the binomial *P. megalacanthum,*—a parasite intimately related to 2 somewhat smaller species, *P. polymastum* Drechsl. and *P. mastophorum* Drechsl., neither of which has hitherto revealed any proliferous tendency in its asexual reproduction (17).

SUMMARY

Decay of watermelons caused by one or another of 9 known species of *Pythium* has been found widely distributed in the United States. In regions where the crop grows and matures under moderately dry conditions, the losses are usually insignificant. Rather substantial losses result, however, in some parts of the Middle Atlantic States, where during wet seasons a late crop is exposed to more abundant infection. Normally, the fungi gain entrance into uninjured fruit at the flower scar, their advance through the massive berry being manifested externally either in a watersoaked appearance or in dark brown discoloration, depending in large part on the identity of the parasite concerned. Descriptive accounts of 3 species producing dark brown blossom-end rot, *P. acanthicum, P. periplocum* and *P. helicoides* are given herein to supplement the diagnoses previously published. Similar discussion is devoted also to *P. anandrum,* one of many species capable of causing decay when inoculated artificially into watermelons, but so far not known to occur spontaneously on fruits in the field.

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THE NOMENCLATURE OF PLANT VIRUSES[1]

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At the last meeting of The American Phytopathological Society at Indianapolis, a committee on virus nomenclature was appointed by the Council. One of the duties of this committee was to arrange a program for discussion of virus nomenclature at this session of the Society. It has fallen to my lot to prepare a general paper on virus nomenclature, as a part of this program. In the preparation of this paper it has not been feasible to consult with other members of the Committee to the extent desired. Therefore, the ideas expressed do not necessarily represent the opinions of the Committee or of any member of it other than myself. This paper is an attempt to present my own picture of the present status of virus nomenclature. If it stimulates discussion helpful, even in a small way, in the solution of some of the problems involved, its full purpose will have been realized.

Characteristically, man throughout his conquest of nature has first designated the new and the unfamiliar by descriptive phrases and later, after becoming better acquainted, has attempted classification and application of specific names. In relation to viruses, plant pathologists have entered the second phase of this conquest and are now beginning to apply specific names to the virus entities with which they have been dealing for a considerable period.

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When the number of virus diseases was relatively small, workers were able to make rather satisfactory progress using descriptive names for the diseases and ignoring for the most part the causal viruses so far as definite terms were concerned.

During these earlier periods, numerous descriptive names designating recognizable diseases came into common usage. Many of these names are still serving a very useful purpose in virus literature. Such terms as "mosaic," "streak," "curl," and "yellows" indicate very definite types of symptoms, but they no longer have any appreciable value in indicating specific diseases caused by distinct agents.

As the number of recognized viruses grew, attempts were made to meet the ever-increasing complexity of virus nomenclature by introducing qualifying adjectives and phrases. As a result there arose innumerable terms such as "mild mosaic," "rugose mosaic," "typical tobacco mosaic," and "virus of apparently healthy potato mosaic."

It became evident with the increased number of names applied to virus diseases, and with the duplication and loose usage that is inevitable with the use of common descriptive names, that if the science of plant virology was to avoid chaos and advance in an orderly manner in the elucidation of the problems presented by this group of diseases, a systematic classification and naming of the causal viruses must of necessity be evolved and adopted to supplement the common names of the diseases already in use.

James Johnson was the first to attempt a comprehensive solution of this problem and to him belongs the credit for much of the progress that has been made. Others, however, have contributed valuable suggestions. It is evident that before specific names can be applied to viruses some system of classification into logical groups and subdivisions must be made available. This search for a logical basis for classification has occupied the attention of a number of persons during the last decade, and numerous suggestions have been made regarding the feasibility of using certain characters for differential purposes. From these suggested characters I have selected, for brief consideration, the following as being of greatest value:

1. Type of symptoms produced on different species and varieties of susceptible plant.
2. Morphological and cytological disturbances produced.
3. Relation of insect vectors to virus transmission.
4. Antigenic reactions in animals and plants.
5. Chemical and physical properties of the viruses themselves.

The first plant viruses described were identified on the basis of symptoms produced on the infected plants. Symptoms have continued to be the chief characteristic by which many of the plant viruses are recognized. It seems certain that symptoms will be important in any attempted classification of viruses and for some time at least symptoms are likely to be the sole character for the identification of a considerable number of plant viruses. Also, reactions on differential hosts are of value in many instances in the separation of closely related viruses or virus strains.

Symptoms have been used as a basis on which to divide virus diseases into the two great groups of "yellows" and "mosaics." The yellows diseases are characterized by yellowing, leaf curling, leaf rolling, and sometimes by dwarfing and rosetting. The mosaic diseases are characterized principally by mottling but sometimes by local lesions.

There are other characteristics of these two groups that have received little or no attention from systematists, but that give support to the idea that this division of the diseases into yellows and mosaics is a logical basis on which to divide viruses into two large groups. For example, the viruses causing the yellows diseases are not readily transmissible by mechanical inoculation, their insect vectors show a high degree of specificity, and often the carbohydrate/nitrogen ratio of leaves of affected plants is relatively high. Whereas, the viruses causing mosaics are often readily transmissible by mechanical means, in many instances the insect vectors are numerous or lack specificity, and often the carbohydrate/nitrogen ratio of leaves of affected plants is relatively low.

There is some evidence that these characteristics result from tissue relationships in which the viruses of the yellows group of diseases are more or less restricted to the phloem and those of the mosaic group occur abundantly in both phloem and parenchyma. The differences enumerated are those that would be expected from such tissue relationships. It is possible that these relationships with the resultant characteristics constitute a basis for a division of viruses into two, more or less, clearly definable groups.

Morphological and cytological disturbances have received a certain amount of attention as characters valuable in virus classification. Quanjer (6) suggested that the viruses of potato should be identified, named, and classified on the basis of morbid effects they produce on a variety of potato that shows clearly dependable internal symptoms. He originated and presented a system of suggested international nomenclature which, however, is essentially a nomenclature of the virus diseases of potato rather than of the causal viruses. As yet, this classification has not extended beyond the virus diseases of potato and it seems doubtful whether the conditions imposed by the system can be met readily in any of the other groups of virus diseases. Morphological and cytological disturbances may, however, be useful in the differentiation of certain viruses that are otherwise difficult to distinguish.

Insect vectors have been very useful in differentiation in certain groups of viruses and Storey (5) has urged a more extensive use of the vector in virus classification. Utilization of the vector in this connection, however, is naturally limited to those groups where the vectors are known to be more or less selective and specific.

The antigenic properties of plant viruses, when introduced into animals, have provided some interesting and valuable information in the identification and separation of certain viruses and virus strains of tobacco. The limits of this method of virus differentiation are not yet known but the method may prove too cumbersome to be utilized extensively in virus classification at least until more easily available methods are developed.

The so-called antigenic reactions of plants in the differentiation of viruses and the indication of relationships between virus strains was stressed by Kunkel (4). This method involves the principle in which, when a plant is infected by one strain of a particular virus, it becomes immune from subsequent infection by other strains of the same virus but retains any susceptibility it previously may have had to infection by unrelated viruses. Evidence supporting this concept has been obtained with certain tobacco, potato, peach, and sugar-cane viruses. There is, however, some question as to whether this phenomenon represents a true antigenic response, and the extent to which it indicates virus relationships remains to be determined. The principle apparently has no value in indicating relationships between strains of the virus of red raspberry mosaic or between strains of the virus of curly top of sugar beet.

Certain physical and chemical properties of viruses such as thermal inactivation point, tolerance to dilution, longevity *in vitro,* and resistance to the lethal action of certain chemicals, are sufficiently constant and independent of the medium in which they occur to permit their use in virus identification. Since physical and chemical properties constitute some of the most distinctive characteristics of viruses, they have been used extensively by Johnson in classification and identification. Unfortunately, properties can be determined only for those viruses that are readily transmissible by mechanical means, or in the rare cases where the viruses, after being subjected to a desired treatment, can subsequently be introduced into the plant through the agency of an insect vector.

From the available differential characters, Johnson and Hoggan (3) selected 5 for use in separating viruses into groups. The characters selected are the following: (1) mode of transmission, including any known insect vectors; (2) natural and differential hosts; (3) longevity of the virus *in vitro;* (4) thermal inactivation point; and (5) certain distinctive and specific symptoms. It is worthy of note, however, that the grouping possible from the use of these characters did not lead to the separation of groups of viruses that could be considered to have natural or artificial affinities or relationships that would justify the designation of any of the subgroups as having the equivalent of generic rank. The excellent key to viruses, developed on the basis of this grouping is essentially, therefore, a key to the individual viruses or to what may be considered the equivalent of species.

This contribution by Johnson and Hoggan emphasized, perhaps better than any other work, the fact that there is yet no satisfactory basis for the grouping of viruses along the lines of their possible natural relationships. It illustrates strikingly the reason why all attempts to designate viruses by a binomial or a trinomial nomenclature have depended on the host plant of the viruses as a basis for generic designation. For this reason, the classifications that have been proposed are essentially types of host indexes to the viruses concerned.

At the present time, it seems probable that the host plant is the most logical basis for generic designation of viruses. It is within the realm of possibility, however, that purification and crystallization of virus proteins may eventually offer a more logical basis for grouping viruses into more natural groups based on characteristics peculiar to the viruses themselves.

With these considerations of the possibilities available in the classification of viruses, we may pass to the specific question of virus nomenclature.

The binomial system is so firmly established in the minds of biologists that it is doubtful whether a system of virus nomenclature that does not include the essentials of the generic and specific concept has a reasonable chance of succeeding. In the present state of knowledge of viruses it is probable, therefore, that the name applied to a virus must consist of at least two terms, one designating a group and corresponding to the genus, and one designating an entity equivalent to a species.

Probably the majority of scientists at the present time are of the opinion that viruses are high molecular weight proteins capable of reproduction in a specialized medium, that medium perhaps being restricted to living protoplasm. Whether viruses are of molecular order in size or larger, or whether they are living or non-living, probably is of no great importance in the present discussion. Perhaps it is sufficient that it can be stated with considerable confidence that in complexity they occupy a position in the realm of organized matter somewhere above the chemical compounds whose structural configurations are known and somewhere below the smallest recognized organism.

In providing names for such entities, therefore, it would seem that reasonable arguments favoring any one of three courses could be advanced. (1) Viruses might be considered organisms and given binomial designations following the practices already in use with recognized organisms. (2) They might be considered chemical compounds and given chemical designations. (3) An arbitrary system having no reference to the nature of the entities might be adopted. Such a system might be designed either as a permanent nomenclature or as a temporary expedient to depend on subsequent discoveries as to the nature of viruses.

Considerable progress already has been made toward the adoption of a system of nomenclature that may be considered as of the third type mentioned above. Johnson (1, 2) presented a system of classification and nomenclature in which viruses are grouped according to their type hosts and numbered chronologically more or less in the order of their discovery.

Thus the first virus described in tobacco becomes "*Tobacco virus 1,*" the second "*Tobacco virus 2,*" etc. The strains are designated by capital letters as for example "*Tobacco virus 1A,*" which would be the type strain of this virus. Other strains are lettered alphabetically, conforming in a descending order as nearly as possible to their degree of virulence. Substrains are designated by small letters, as, for example, "*Tobacco virus 1Bc*" would indicate a substrain of "*Tobacco virus 1B.*" This system was favorably considered by the Committee on Description and Nomenclature of Plant Viruses appointed by the Fifth International Botanical Congress in 1930 and was adopted "in principle" by this Committee at the Sixth International Botanical Congress held in Amsterdam in 1935.

Since 1927, when Johnson (1) introduced the basic principles of this system of nomenclature, numbering of viruses has been extensively practiced. Each investigator, however, with one or two exceptions, has had a new and different concept of the terms that should precede the numbers and letters used to designate the equivalent of species and strains. Thus we have such designations as "Curl virus 1" of raspberry, "Bean mosaic virus 2," "Cucumber mosaic virus strains 1, 2, 3, 4," and "Bean mosaic 3." These types of numbering have contributed to the differentiation and recognitions of the components of certain limited groups of viruses but it is doubtful whether they have been correspondingly important in the development of a uniform virus nomenclature.

With certain modifications, the system of nomenclature introduced by Johnson was adopted by Kenneth Smith (7) in a recent extensive classification of viruses in which some 144 viruses, exclusive of strains, were named.

In this classification, the Latin generic name of the host plant is substituted for the common name used by Johnson.[2] Thus "*Tobacco virus 1*" becomes "*Nicotiana virus 1*" with similar changes with viruses of other plants. Strains are indicated by capital letters, as in Johnson's system, except that lettering begins with the strains derived from the type and the type strain has no letter The order of lettering has no relation to virulence of the strains or to any other indicated character so far as I can detect.

Furthermore, the order in which the viruses of some of the groups are numbered departs widely from that suggested by Johnson, but the basis for this departure is not clearly indicated in all instances. Part of the difference is due to a difference in concept as to what constitutes valid entities. For example, the virus of yellow mosaic of tobacco is "*Tobacco virus 6*" in Johnson's (1) classification; but Smith considers that this virus is a strain of ordinary tobacco mosaic virus and designates it "*Nicotiana virus 1A.*"

It is obvious, of course, that when a numbered virus of a series is reduced to the rank of a strain, further shifting of other viruses is necessitated if all of them are to be numbered serially. This may partially account for the extensive renumbering that occurred in some of the groups, particularly in the tobacco group, where "*Tobacco viruses 8, 11, 12, 13,* and *18*" as proposed by Johnson[3] become *Nicotiana viruses 4, 5, 6, 7,* and *8,* respectively, of Smith.

In a consideration of the establishment or the stabilization of any system of virus nomenclature it must be recognized that virus numbering is rather firmly established in the literature dealing with plant virus diseases. In fact, nearly all of the viruses have been numbered at least once, and several have acquired a respectable synonomy under this system. For example, the virus of common pea mosaic, according to Smith, may now be called "*Pisum virus 2,*" "*Pea virus 1,*" or "*Pea virus 3.*"

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[2] Johnson (1, p. 5) also suggested the possibility of using the generic name of the host plant and stated, "In naming the virus, it may be preferable in some or all cases to use the Latin generic name or binomial in place of the common name of the host plant."

[3] *Tobacco viruses 11, 12, 13,* and *18* appear in a mimeographed list prepared by Johnson, "Illustration of proposed system of nomenclature for plant viruses." The names presented in this list are given as synonyms by Smith (7).

It would seem that two courses are open to further action in the development and perfection of virus nomenclature. Either the essentials of the practices now in limited use, but already extensively applied may be unified and clarified to permit their general acceptance, or a new and different system of virus nomenclature must be considered.

If the former course is decided upon, it is important that an early agreement be reached as to the basis for a uniform usage if further confusion of names is to be reduced to a minimum.

At present, there seems to be general acceptance of the host plant as a basis for generic designation. It would seem that agreement as to the details of the generic equivalent to be selected could be reached without any great amount of difficulty.

Also, up to the present time, there has been more or less general agreement that a number should be used to represent the equivalent of a species. In practice, however, the lack of uniformity of usage and lack of adherence to the principle of priority have given ground for serious concern regarding the practicability of the use of numbers to designate species.

In this capacity numbers have some decided disadvantage, which should be considered in any attempt to establish a system of nomenclature.

First, the simplicity of the numbering system may prove to be one of its sources of greatest confusion. Numbering viruses is so easily accomplished that it may be that it lends too much encouragement to frequent changes in numbers and to the numbering of viruses that are inadequately characterized to permit accurate identification.

Second, a number means nothing in respect to any characteristic of a virus, or of the disease it causes. For this reason, numbers are somewhat difficult to remember in association with specific viruses.

Third, numbers do not permit the desired degree of mobility in the organization of viruses according to different concepts of relationships. For example, the virus of curly top in North America is designated by Smith as "*Beta virus 1.*" The virus of curly top in South America, which produces identical symptoms on beets, but is transmitted by a different insect vector, is also designated "*Beta virus 1.*" It is by no means certain that the second virus is identical with the first. If the two viruses prove to be distinct, the South American virus must become "*Beta virus 6,*" since the intervening numbers have already been assigned to other viruses, some of them of the mosaic type. If this occurs, it is obvious that in any general treatise on virus diseases of beet they must be arranged out of order as to number of causal virus or out of order as to similarity of symptoms produced.

As a somewhat different example, we may cite the case of "*Tobacco virus 5,*" one of the first viruses numbered. Later, it was found that this virus is "*Potato virus 17.*" In this instance, what is to become of the number "6" in relation to subsequent numbering of the viruses of tobacco? Current practice in biological nomenclature would demand that this number be discarded for all future use as a specific designation of any virus of tobacco. This, in some ways, is undesirable since the viruses of tobacco can no longer be numbered serially. This may lend encouragement to the practice of shifting numbers in order to satisfy concepts as to logical orders of arrangement of viruses.

It is quite obvious, of course, that some of these difficulties, and perhaps the most important ones, are possible of solution by agreement.

However, if before agreement is reached the numbering system becomes hopelessly confused through lack of coordination, it would seem that before any new system is introduced for serious debate it would at least be worth while to consider the advisability of substituting names for the numbers in the systems already in limited use. It seems to me that names might effectually solve some of the difficulties and they might even have certain advantages not possessed by numbers. Names, especially if well chosen, would be easier to remember in association with viruses; they might be less subject to frequent change, and perhaps there would be less incentive to apply names to viruses that are imperfectly characterized.

Under such a modification the basic structure of the general system now in use would be preserved. Names would simply replace numbers. For example, "*Tobacco virus 1*" of Johnson might become "*Tobacco virus altathermus*" or "*Nicotiana virus altathermus.*" If subsequent investigation discloses that viruses are living organisms and it becomes advisable to use the Latinized binomial system of nomenclature, this virus with its specific name would be assigned to its proper genus, in which case it might become "*Paracrystalis altathermus.*" If, on the other hand, it is found that viruses are true chemical compounds and if, therefore, a chemical designation is deemed desirable, the specific name would become the chemical name probably modified by an appropriate suffix.

The designation of certain classes of chemical compounds by a common suffix is an established practice in chemistry, as, for example, the suffix "ase" to designate enzymes. In a similar manner, it might be found desirable to adopt a suffix, perhaps "vir," to designate a virus. In this case "*Tobacco virus 1*" would become automatically "altathermovir."

Perhaps it is needless to state that none of these terms are proposed for adoption, but are used solely for the purpose of illustration.

It is my conviction, however, that the future of virus nomenclature is dependent to a much greater extent on international agreement as to common practices and common usages than it is on the perfecting of a particular system. It is evident that up to the present time no system of virus nomenclature has been accepted by a sufficient number of virus workers to ensure its permanent adoption and use. No system seems likely to receive such acceptance until more definite action is taken to establish uniform rules, or a code—governing its operation.

Further research will undoubtedly add enormously to our knowledge of viruses and may be expected to provide information that, if available at this time, would furnish a more satisfactory basis for classification than any we now have. However, it is doubtful whether it would be wise to consider delay in classification in anticipation of this result; in fact, even were such delay desirable, we probably no longer have that choice. The fact must be accepted that viruses are going to continue to be classified and named. We have only the choice of whether classification and nomenclature shall proceed along an individualistic path in which each investigator classifies and names the viruses with which he is working, according to a system that appeals to him at the moment, or whether virus nomenclature shall proceed in accordance with a generally accepted system operating under uniform rules of usage.

It would seem, therefore, that we have reached the stage in the study of plant viruses where it is important to encourage free discussion of the problems of virus nomenclature not only from the viewpoint of the merits of the various systems that have been proposed or that may be formulated, but also from the viewpoint of the problems to be solved in obtaining general acceptance of some one system, and the problems involved in formulating a code under which the accepted system shall operate.

When the possibilities of the various proposals are thoroughly explored there is hope that a common ground may be found on which some representative group of virus workers may be able to crystallize a system of nomenclature that will be generally acceptable and will place virus nomenclature on a basis of uniformity and stability comparable to that attained in the nomenclature in other branches of biology.

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PROPOSAL FOR EXTENSION OF THE BINOMIAL SYSTEM OF NOMENCLATURE TO INCLUDE VIRUSES

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It may seem unnecessary to propose at this time a plan for naming viruses, in view of the contemporary use of several systems of nomenclature. There are, however, certain features of the present systems that may prove incapable of meeting the demands of future developments. Progress in understanding viruses and their relationships demands a persistent attempt to classify in a logical manner, as well as to name. Early attempts at virus nomenclature did not meet this need, which, indeed, hardly existed until recently. Today, there is a growing recognition among virologists of the value of logical classification as an adjunct of nomenclature.

For some years the problem of how to designate phytopathogenic viruses and their strains has been actively discussed. Johnson, a pioneer in this field, early developed a method of nomenclature (3, 4, 5, 6) that made use of the common names of principal host species followed by serial numbers. Johnson's method has been widely followed. Quanjer (10) subsequently proposed a method of naming viruses and viroses of the potato. This method was based on symptom expression only, and has not been used extensively. Smith (11), in a recent textbook on virus diseases of plants, introduced a modification of Johnson's numerical system, in which the scientific name of the genus replaced the common name of the host species. The two numerical systems of nomenclature have in common the disadvantage of grouping together unlike viruses that are found in the same host. There is little reason for grouping viruses with reference to any particular one of their usually numerous hosts. The two systems are essentially linear accession lists, arbitrarily subdivided for convenience. The groups that have been named from common host plants unfortunately conceal rather than display the fundamental virus relationships that gradually are becoming known. Moreover, they now bring confusion because designating numbers have become large in some groups. The numbers no longer readily call to mind even the better known of the individual viruses. In addition to these principal objections to the numerical systems of nomenclature, there are two minor objections: first, elimination of any number that has been assigned to a supposedly new virus that subsequently is found to be a strain of a previously known virus causes discontinuity in the list, which becomes confusing in later summaries or monographs; and second, the use of serial numbers tends to suggest closeness of relationship between viruses assigned successive numbers, although such an implication is unintentional.

The success of the more or less descriptive Latinized binomials now universally used in the nomenclature of plants and animals suggests an extension of this system to include viruses. There have been some earlier attempts at binomial nomenclature of viruses. In several of these cases, however, the binomials were applied originally to entities not now recognized as being the causative viruses of the diseases in question. Thus, Palm (9) used the name *Strongyloplasma iwanowskii* for granules not now believed to represent tobacco-mosaic virus; McWhorter gave the name *Phytamoeba sacchari* to an amoeba no longer believed to represent the etiological agent of Fiji disease of sugar cane (8); and d'Herelle (1) used the names *Bacteriophagum intestinale* and *Protobios bacteriophagum* to represent visible particles from lysed bacteria. Other binomials are perhaps applicable to the causative viruses of specific diseases; examples are *Strongyloplasma avium, S. ovinae, S. vaccinae, S. hominis, S. variolae, S. paravaccinae* applied by Lipschütz (7) to the elementary bodies of fowl pox, sheep pox, vaccinia, molluscum contagiosum, variola, and paravaccinia, respectively, and *Sanarellia cuniculi* applied by Lipschütz (7) to "sanarellien" in myxomatosis of rabbits. The *Borreliota* species named by Goodpasture (2) appear to be synonymous with the *Strongyloplasma* species of Lipschütz.

Recent studies have shown that, in regard to particle size, viruses form a graded series between recognized microorganisms and macromolecules of plant and animal origin. There is little, if any, evidence of discontinuity in the series from simplest virus to obviously living microbe. The members of this series share with higher biologic forms ability to multiply and to produce variants, and appear unable to arise save by a prolonged process of evolution. It is not essential to classification that the exact relationships of viruses to the plant, animal, and mineral kingdoms be understood at present. For taxonomic purposes, the viruses may be considered as a distinct group. Their own interrelationships, as shown by studies of derivation of strains and by serological and immunological comparisons, are suggestive of divisions into groups that can be portrayed readily by a system of nomenclature similar to that used by Linnaeus in naming and classifying the higher biologic forms.

The following system of classification by binomials is suggested for the consideration of all those who are interested in the organization of knowledge about filterable viruses. If it should prove acceptable, its use may well be delayed, to avoid confusion in the literature, until there has been time for thorough criticism by all concerned. It is hoped that the suggested system may be an improvement over currently used numerical schemes, despite its biological connotation and the bizarre impression that may be conveyed at first by new names. Several advantages may be claimed for it. Grouping is based on fundamental similarities between viruses, so far as these have been disclosed by the results of serological and immunological tests, similarity of induced diseases, and data accumulated from other studies of virus properties. Names suggestive of what is known of the viruses have been chosen where possible. New virus groups, new viruses, and new strains may be introduced into this system at any point, in the way well understood in connection with the nomenclature of plants and animals.

Names no longer found applicable may be dropped without affecting the continuity of the system. No attempt has been made to include all known viruses in the present list, but representative ones have been chosen.

Some virologists may hesitate to use a scientific name for a virus not yet isolated from host tissues and observed in a state of purity. Yet they are willing to adopt a common name for such a virus. The common name implies distinctiveness of the virus entity as much as does a Latinized binomial, which has the added advantage of international acceptability. Quanjer (10, p. 608) has wisely pointed out that form, the usual basis of nomenclature in the vegetable and animal kingdoms, is but a function of chemical processes. Typical injuries caused by the presence of specific viruses in their hosts are diagnostic in the same sense and are also expressions of chemical processes. It would appear to be sound practice to base nomenclature on characteristic function whether that function is perceived through its determination of individual form or through its influence on the life processes of host organisms.

The desirability of the binomial form of nomenclature for viruses should be considered independently of the appropriateness or lack of appropriateness of the actual virus groupings here suggested. Plants and animals have been studied much longer than viruses, yet revisions of genera and larger groups are still occasionally necessary, even with them. Rearrangements of the present tentative groupings, formation of new groups, and perhaps partition or elimination of old groups may be needed from time to time and can be put into effect whenever justified by the results of appropriate investigations. Binomial nomenclature, though recognized to have many faults, has proved its flexibility and value for representing changing views of natural relationships in the past. Its extension to viruses should prove a considerable convenience for portraying current classifications.

PROPOSED CLASSIFICATION OF VIRUSES

Kingdom *VIRA*[1]	Viruses
Division I. PHYTOPHAGI	Viruses parasitic in plants; phytophages
Class I. *SCHIZOPHYTOPHAGI*	Viruses parasitic in schizophytes; schizophytophages
Family 1 PHAGACEAE	Viruses parasitic in bacteria; bacteriophages
Genus *Phagus* (from Gr. φαγός, from φαγεῖν, to eat)	
P. minimus, type sp.	Bacteriophage S13
P. parvus	Bacteriophage C13
P. dysenteriae	Bacteriophage D13

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[1] The Latin *virus*, a second declension neuter noun meaning poison, slime, or stench, was not used in the plural in the Classical Latin texts. I am indebted to H. H. Bender of Princeton University for the opinion that the choice of *vira* as a New Latin neuter plural is more easily defensible than that of *viri*, which is masculine in form and would be confused with Latin *viri*, men, or that of *vire*, which might be formed after Latin *pelage* and *cete*, both of which are Greek plurals for loan words in Latin; *cf.* also Latin *venena* (second declension neuter plural), which was substituted as plural of *virus*.

P. astrictus	Bacteriophage D3
P. coli	Bacteriophage C21
P. maximus	Bacteriophage D4
Class II. *SPERMATOPHYTOPHAGI*	Viruses parasitic in flowering plants; spermatophytophages

Family 1. CHLOROGENACEAE. Yellows group; viruses causing diseases mostly characterized by stimulation of normally dormant buds to form witches'-brooms, by chlorosis without spotting, or by both brooming and chlorosis. Invaded parts usually abnormally erect. Vectors typically leaf hoppers (Jassidae).

Genus *Chlorogenus* (from Gr. χλωρός, light green or yellow + gen, suffix signifying producing, from Gr. γένος, descent)

C. callistephi, type sp.	Aster-yellows virus
var. *vulgaris,* type var.	Typical strain of aster-yellows virus
var. *attenuatus*	Heat-attenuated strain of aster-yellows virus
var. *californicus*	Celery-yellows strain of aster-yellows virus
C. persicae	Peach-yellows virus
var. *vulgaris,* type var.	Typical peach-yellows virus
var. *micropersica*	Little-peach virus
C. rosettae	Peach-rosette virus
C. solani	Potato witches'-broom virus
C. santali	Sandal-spike virus
C. vaccinii	Cranberry false-blossom virus
C. robiniae	Locust witches'-broom virus
C. fragariae	Strawberry witches'-broom virus

Family 2. MARMORACEAE. Mosaic group; viruses causing diseases usually characterized by persistent chlorotic or necrotic spotting, and often by mottling; no stimulation of normally dormant buds; usually no recovery; if recovery occurs, no immunity from reinfection. Vectors, typically aphids (Aphididae), sometimes thrips (Thysanoptera), or leaf hoppers (Jassidae).

Genus *Marmor* (from L. *marmor* n., a mottled substance, marble)

M. tabaci, type sp.	Tobacco-mosaic virus
var. *vulgare*	Green-mottling, distorting strain
var. *aucuba*	Tomato aucuba-mosaic strain
var. *obscurum*	Masked-symptom strain
var. *deformans*	Tomato enation-mosaic strain
M. cucumeris	Cucumber-mosaic virus
var. *vulgare,* type var.	Common cucumber-mosaic virus
var. *upsilon*	Potato vein-banding strain
var. *commelinae*	Southern celery-mosaic strain
var. *lilii*	Lily-mosaic strain
M. dubium	Potato-mottle, or X virus

var. *vulgare*, type var.	Mottle virus proper
var. *annulus*	Potato-ringspot strain
var. *obscurum*	Masked-mottle strain
M. erodens	Tobacco-etch virus
var. *vulgare*, type var.	Etch virus proper
var. *severum*	Severe-etch strain
M. solani	Potato mild-mosaic virus
M. abutilon	Abutilon-mosaic virus
M. aucuba	Potato aucuba-mosaic virus
M. maidis	Maize-streak virus
M. persicae	Peach-mosaic virus
M. sacchari	Sugar-cane mosaic virus
M. pisi	Enation mosaic of pea
M. phaseoli	Bean-mosaic virus
M. tritici	Wheat-rosette virus

Family 3. ANNULACEAE. Ringspot group; viruses causing diseases characterized by necrotic or chlorotic spotting with concentric-ring lesions; eventual recovery with nonsterile immunity. No insect vectors known.

Genus *Annulus* (from L. *annulus* m., a ring)

A. tabaci, type sp.	Tobacco-ringspot virus
var. *virginiensis*, type var.	Typical strain
var. *kentuckiensis*	Green-ringspot strain
var. *auratus*	Yellow-ringspot strain
A. zonatus	Tobacco-ringspot 2 virus

Family 4. GALLACEAE. Fiji-disease group; viruses causing diseases characterized by proliferation of normally inactive tissues; chlorotic and necrotic mottling absent; witches' brooms, if formed, not of spindly shoots.

Genus *Galla* (from L. *galla* f., a gall nut)

G. fijiensis, type sp.	Fiji-disease virus

Family 5. ACROGENACEAE. Spindle-tuber group; represented by a virus causing disease characterized by abnormal growth habit, without chlorotic or necrotic mottling, systemic chlorosis, or witches'-broom formation.

Genus *Acrogenus* (from Gr. ἄκρον, point or peak + gen, suffix signifying producing, from Gr. γένος, descent)

A. solani, type sp.	Potato spindle-tuber virus

Family 6. RUGACEAE. Leaf-curl group; viruses causing diseases characterized by arrested development of invaded leaf tissues, resulting in leaf curl, enations, and other deformities. Vectors, typically whiteflies (Aleyrodidae).

Genus *Ruga* (from L. *ruga* f., a wrinkle)

R. tabaci	Tobacco leaf-curl virus
R. gossypii	Cotton leaf-curl virus
R. bemisiae	Cassava-mosaic virus

Division II. ZOOPHAGI[2]	Viruses parasitic in animals
Class I. *ARTHROPODOPHAGI*	Viruses parasitic in arthropods
Class II. *CHORDATOPHAGI*	Viruses parasitic in chordates

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THE USE OF CALCIUM CYANAMID FOR THE DESTRUCTION OF APOTHECIA OF SCLEROTINIA FRUCTICOLA

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INTRODUCTION

The destruction of fruit mummies by several different methods has been recommended as part of the program for the control of brown rot on stone fruits caused by *Sclerotinia fructicola* (Wint.) Rehm. However, few orchardists in the prune area of southwestern Washington follow any of these methods. Most growers, especially those handling large acreages, are adverse to the collection and destruction of affected fruit during and immediately following harvest because of the amount of labor and expense involved. Because of the high soil-moisture content usually prevalent during winter and spring, the practice of plowing mummies under and destroying apothecia by harrowing is often difficult, as many orchards are located on soils not readily worked until the period of apothecial production is over. It is generally believed that prune mummies may survive turning under and produce apothecia when placed on or near the soil surface by subsequent plowing.

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[2] Names for viruses having animal hosts may best be recommended by animal pathologists, but, for the sake of showing relationships between them and viruses affecting cryptogams and phanerogams, classes are indicated here. This, incidentally, serves to emphasize the fact that the viruses affecting unicellular plants (bacteria) have as yet no known counterpart capable of affecting unicellular animals (protozoa).

Isolations made just prior to blossoming in 1937 and 1938 revealed that less than 2 per cent of over-wintering mummified fruits hanging on the trees and showing fungous sporulations, and none overwintering on the ground, produced cultures of *Sclerotinia fructicola.* The present program includes a preblossom spray of lime-sulphur, which should destroy the few viable conidia of the organism present in the orchard. The destruction of the ascigerous stage should, therefore, play an important part in the control of the disease.

Although a number of soil treatments have been used in combating seed and soil-borne organisms, only a few have been used to prevent the development of the overwintering or ascigerous stages of parasites of aerial organs developing on or near the soil surface. Keitt and Palmiter (5) found that spraying apple leaves on the ground beneath trees with a solution of ammonium sulphate killed the mature ascospores of *Venturia inaequalis* and prevented the maturation of others. Holz (4) found that the development of perithecia of *V. inaequalis* was entirely checked in pot experiments when apple leaves were treated with calcium cyanamid. It occurred to the writers that calcium cyanamid (3, 6) might inhibit the development of apothecia on prune mummies; consequently, an experiment was conducted to determine if this could be accomplished.

EXPERIMENTAL METHODS AND RESULTS

Commercial pulverized and oiled calcium cyanamid was applied on April 5, 1938, to the soil already showing some apothecia and to the light vegetative cover of Austrian field peas and chickweed, *Stellaria media* (L) Cyrill., at the rate of 220 pounds per acre; (a) broadcast by hand under 3 trees; (b) applied with a knapsack duster under 3 other trees. Twenty-four hours later the exposed apothecia had become discolored and somewhat shriveled and the vegetative cover showed evidence of burning. Within a few days the apothecia had dried (Fig. 1) and the vegetative cover had turned dark and decomposition had set in.

Measured areas of treated and nontreated soil under the trees were carefully examined for apothecia several times during the period of apothecial development.[1] The results are presented in table 1.

Only one viable apothecium was found on the treated area during this period (Table 1). It was located in an area a few inches in diameter where the vegetative cover had not been injured, indicating that the calcium cyanamid had not been distributed evenly by hand broadcasting. Observations made throughout the summer and fall showed no injurious effects to the trees under which the calcium cyanamid had been applied.

[1] Apothecial activity was observed from April 4 until April 27.

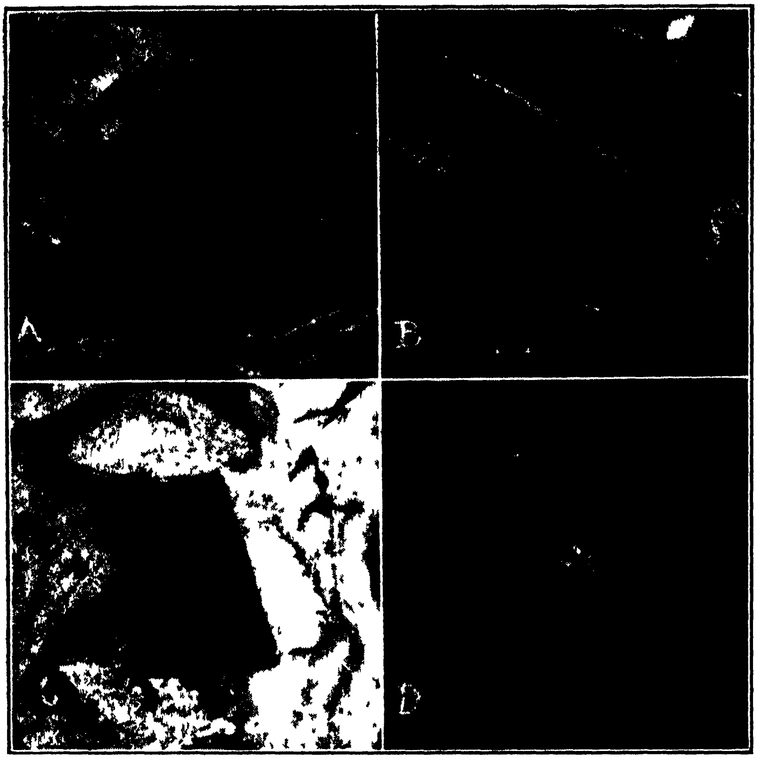

FIG. 1. Apothecia of *Sclerotinia fructicola*. A and C. Normal apothecia. B and D. Apothecia burned by calcium cyanamid. Natural size.

An additional study of the action of calcium cyanamid on development of apothecia was made in which wooden boxes 12 × 12 × 8 in., previously filled with orchard surface soil (Felida silt loam), were used. They were sunk into the soil under trees so that the top of the soil in the boxes approximated that of the soil in the orchard. Fifty brown-rot prune mummies, collected on May 17, 1937, from the 1936 crop, were worked into the soil in each box. On April 7, 1938, calcium cyanamid was applied at the rate of 324

TABLE 1.—*The result of soil treatment with calcium cyanamid on production of apothecia*

Treatment	Date applied	Method of application	Area examined (sq. ft.)	Date examined	No. viable apothecia
Calcium cyanamid (220 lb. per acre)	April 5	Duster	64	April 11	0
None			64	April 11	68
Calcium cyanamid (220 lb. per acre)	April 5	Broadcast	36	April 15	1
None			36	April 15	35
Calcium cyanamid (220 lb. per acre)	April 5	Duster	36	April 18	0
None			36	April 18	38

pounds per acre to the surface of the soil in each of 2 boxes. Two other nontreated boxes served as checks. Several apothecia and a vegetative cover of chickweed were present in each box at the time of treatment. Ten days later an average of 27 viable apothecia were found in each of the nontreated boxes, while none were found in the treated boxes. The vegetative cover in the treated boxes was completely destroyed.

DISCUSSION

In this experiment calcium cyanamid was found to be effective in the destruction of maturing apothecia of *Sclerotinia fructicola* and in preventing the formation of others. Several investigators (1, 2, 3, 4) have discussed the toxicity of calcium cyanamid, or its decomposition products, to seeds and certain fungi. Crowther and Richardson (2) are of the opinion that either the cyanamid itself or its acid salt is responsible for the toxic action. They showed that high temperature, low soil moisture, and thorough incorporation of the material with the soil shortened the period of toxicity and that lower temperatures, higher soil moisture and surface concentration of the cyanamid slowed the process of decomposition and increased the period of toxicity.

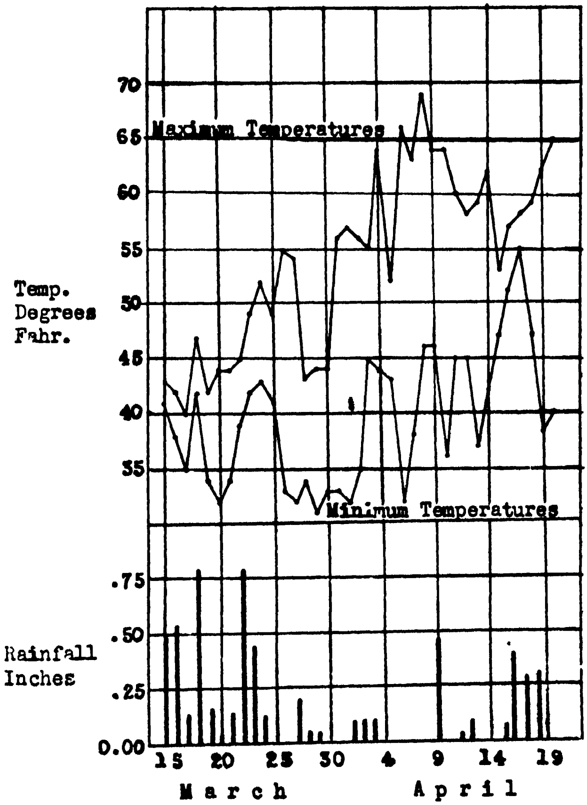

FIG. 2. Maximum and minimum air temperatures and rainfall at Vancouver, Washington, 1938.

Since apothecial development continues over a period of several weeks it is desirable that the period of toxicity be extended over as much of this period as possible. The temperature and soil moisture encountered and the method of application of the material in this study tended to produce an extended period of toxicity. Maximum and minimum air temperatures are given for the period of this study, since soil-temperature data were not obtained (Fig. 2). Smith (7) has discussed the lag of soil temperatures behind those of the air. The average temperature of the area during the period of this investigation was below the lowest used by Crowther and Richardson (56° F.) and the normal field moisture capacity of the soil was considerably greater than any used in their studies. Rainfall data (Fig. 2) indicate that the soil moisture under the trees was close to or at normal capacity. Incorporation of the material with the soil was avoided by applying the material as a dust to the surface of the soil.

Commercial pulverized cyanamid can be applied to the soil either by hand or with a power duster; the latter with either a single or with multiple outlets will give excellent distribution. A small five-row machine delivers 100 pounds of dust every 22 minutes. This means that the quantity of material used per acre in this study can be applied in about 50 minutes.

In addition to the use of calcium cyanamid for the destruction of the apothecial stage of *Sclerotinia fructicola,* the utilization of the nitrogen in the material must be considered in an orchard-management program. Crowther and Richardson (2) have shown that nearly all soils contain the catalysts necessary for the conversion of the nitrogen of calcium cyanamid to urea.

The Felida silt loam used in this study consists of 42.5 per cent silt, 24.5 per cent clay and 33 per cent sand, has a pH of 5.9, and contains a moderately high reserve of more or less readily soluble iron. Crowther and Richardson (2) state that in slightly acid solutions calcium cyanamid is hydrolyzed to urea and the reaction is catalyzed by many inorganic compounds, including certain zeolites and especially the salts or oxides of iron and manganese, which are found for the most part in the finer soil separates. Therefore, it appears that the soil conditions were favorable for the utilization of the nitrogen in the calcium cyanamid.

SUMMARY

Commercial pulverized and oiled calcium cyanamid, applied with a knapsack duster to the surface of the soil and vegetative cover under prune trees at the rate of 220 pounds per acre at the beginning of apothecial production, destroyed apothecia of *Sclerotinia fructicola* and prevented the development of others. Similar results were obtained when soil in wooden boxes, in which apothecia were developing, was treated with a surface application of calcium cyanamid at the rate of 324 pounds per acre.

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TEMPERATURE RELATIONS OF TOBACCO-MOSAIC VIRUS AND ITS HOST

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The masking of symptoms when a virus-infected plant is grown at relatively high temperature is a common characteristic of several mosaic diseases. It was first reported by Johnson[1], who also demonstrated that virus is present in such symptomless leaves. The writer has shown[2] that a similar masking takes place when the host plant is grown at a relatively low temperature. Symptoms of tobacco mosaic, for example, appear between the limits 50 and 98° F., but the temperature range of growth of the host plant is wider. The investigation here reported was planned to discover whether the virus has the same temperature of optimum activity as its host, or whether it has a different relation. The general plan was to measure the rate of movement of the virus, and compare it with the relative rate of growth of the host plant. Such comparisons were made at different temperatures, at first in the approximate temperatures provided by greenhouses variously heated, and later by the more exact conditions of constant temperature chambers. Controlled artificial illumination, and the maintenance of complete humidity, rendered temperature the only variable factor.

EXPERIMENTS AND RESULTS

Low-temperature Masking of Symptoms

The confirmation of two further seasonal observations can now be added to the original account.[2] A cool greenhouse at the Tolson Memorial Museum, Ravensknowle, Huddersfield, normally maintains a temperature of approximately 48° F. during the winter. Ten tobacco plants showing symptoms were transferred to this house in October, 1936, and the experiment was repeated in October, 1937. New growth appeared slowly, but was invariably free from symptoms in both seasons (Fig. 1, A).

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[1] Johnson, J. The relation of air temperature to certain plant diseases. Phytopath. 11: 446–458. 1921.

[2] Grainger, J. Low temperature masking of tobacco mosaic symptoms. Nature 137: 31–32. 1936.

FIG. 1. A. Leaf from tobacco plant grown at 48° F Symptoms are completely masked. B. Leaf from tobacco plant grown at 55° F., with slight symptoms.

A warmer house, maintained at approximately 65° F. adjoins the cool house mentioned above Symptoms there appeared upon all the leaves of diseased plants throughout the winter season. The natural illumination of both greenhouses is the same, so that the masking of symptoms cannot be an effect of light duration or intensity. Plants destined for these experiments were transferred to 6-in. pots, so that root action was not restricted.

Rate of Spread of the Virus at Different Temperatures

Inoculations were made at the tips of three leaves upon each plant. A transverse scratch with the virus-bearing needle limited the inoculated area. Many plants were so treated, and after a certain prearranged time the inoculated leaves were severed with sterile scissors at varying distances from the limit of infection. Batches of three or four plants received identical treatment. Their subsequent state of health indicated whether the virus had traversed the distance from inoculation to cut in the stated time. Plants were always placed under the required conditions at least three days before any treatment was given.

A preliminary test, where experiments on the rate of spread of the virus were performed at varying temperatures with natural illumination, yielded the results set forth in tables 1 and 2.

TABLE 1.—*Distance travelled within host plant by the tobacco mosaic virus in three days*

Distance from inoculation to cut, in cm.	Average temperature in degrees F.					
	108	95	85	75	70	65
	No. of plants diseased, out of 3 inoculated					
1	0	1	3	3	2	0
2	0	0	2	1	3	0
3	0	0	1	1	2	0
4			0	0	0	...

TABLE 2.—*Distance travelled within host plant by the tobacco-mosaic virus in two days*

Distance in cm. from inoculation to cut	Average temperature in degrees F.		
	95	85	65
	No. of plants diseased, out of 3 inoculated		
1	0	2	0
2	0	0	0
3	0	0	0

A similar experiment indicated that the virus had not spread so far as 1 cm. from the inoculation, at 80, 70, and 62 degrees F., when the cuts were made 24 hours after infection.

Two constant-temperature chambers were arranged to run at 85° F. and at 75 or 70° F., since these values seemed to provide an optimum for virus activity, under Huddersfield conditions. The maximum deviation from the desired temperature was ± 2° F. Evaporation of water from earthenware saucers, and from the plant pots always maintained a completely moist atmosphere, and water of condensation invariably appeared upon the glass parts of the chambers. A 100-watt lamp with suitable reflector was suspended above the glazed roof of each chamber, so that the bulb was 2 in. above the glass, and about 8 in. from the plants. Both lights were switched on automatically from 6 a.m. until 10 p.m., and thereafter were extinguished until 6 a.m. the next morning. The chambers were housed in a darkened hut. With light and humidity standardized, leaving temperature the only variable, the results in table 3 were obtained.

No significant difference in the rate of spread of the virus can be observed for the temperatures mentioned, and observations upon the length of time between inoculation and the first appearance of symptoms (Table 4) give a general confirmation to this view.

TABLE 3.—*Distance travelled by the tobacco-mosaic virus within its host in different periods of time*

Period and dist. (in cm.) from inoculation to cut	Results at indicated temperature in degrees F.					
	70		75		85	
	Plants inoculated	Plants diseased	Plants inoculated	Plants diseased	Plants inoculated	Plants diseased
30 hours						
3			4	2	4	2
4			4	0	4	0
5			4	0	4	0
31 hours						
2	4	4			4	4
4	4	1			4	2
5	4	0			4	0
6	4	0			4	0
41 hours						
2	3	3			3	3
4	3	3			3	3
6	3	0			3	0

TABLE 4.—*Length of time between inoculation of host and first appearance of symptoms of tobacco mosaic (incubation period)*

Date of experiment	Temperature in degrees F.	
	85	70
Dec. 1935	7 days	7 days
Sept. 1936	9 days	11 days
Sept. 1936	8 days	9 days

There is, if anything, a slightly shorter incubation period of the virus at 85° than at 70° F.

Rate of Growth of the Host Plant at Different Temperatures

A preliminary estimation of the rate of growth of healthy tobacco plants growing at various temperatures in greenhouses with natural illumination was made. Daily measurements of increase in length of the leaves yielded the results shown in table 5, and comparable figures also were obtained for breadth of the same leaves.

TABLE 5.—*Rate of growth of healthy tobacco plants*

	Average temperature in degrees F.					
	108	95	85	70	60	50
Average increase in length of leaves per day, in mm.	Trace	2.0[a]	4.2[a]	5.6[a]	3.3[a]	0.5[a]

[a] Each figure is an average of 150 measurements spread over 20 days.

A definite optimum is revealed at 70° F., with substantially less growth at 85° F. This is different from virus activity, which was the same at both temperatures (Tables 1 to 3). It therefore became necessary to study the relative growth at these temperatures, under the more exact conditions provided by the constant temperature chambers. Results of such experiments are portrayed by table 6.

TABLE 6.—*Rates of growth of healthy and mosaic-diseased tobacco plants*

	Healthy (20 days)		Diseased (24 days)	
	85° F.	70° F.	85° F.	70° F.
Average daily increase in length of leaf, in mm.	4.9	6.6	4.4	5.6
Average daily increase in breadth of leaf, in mm.	2.5	3.0	2.0	2.4
No. of leaves produced by 5 plants after inoculation	36	35	25	34
Total area of the leaves, in sq. cm.	394	822	423	839
Total increase in dry weight of the shoot, in g.	1.17[a]	1.93[a]	0.64[a]	1.38[a]

[a] These figures were obtained after the deduction of 0.17 g., representing the average dry weight of the shoot at the beginning of the experiment.

The results of table 6 show a definite increase in growth, by whatever criterion it is judged, at 70° F., as compared with 85° F. It is in marked contrast to the virus activity, which was apparently equal at both temperatures (Table 3).

Determinations of total nitrogen and carbohydrate fractions of diseased material grown at the two temperatures (Table 7) suggest that the relatively small increase in dry weight at 85° F. (Table 6) does not seem to fall substantially upon any fraction at the expense of another. The differences, in the order of 3 per cent, between the contents of insoluble and total carbohydrate fractions (Table 7) are barely significant.

TABLE 7.—*Analyses of diseased material*

All figures as percentage of the dry weight	85° F.	70° F.
Total nitrogen 1	4.84	4.88
2	4.51	4.30
Total soluble sugars	6.02	5.56
Reducing sugars	1.42	1.96
Insoluble carbohydrates	6.75	9.88
Total carbohydrates	12.77	15.44

Material was prepared for analysis by drying at 100° C. for half an hour, and then to constant weight at 65° C. Soluble constituents were extracted by 95 per cent alcohol, acting for at least 24 hours. The total nitrogen was estimated by a modified micro-Kjeldahl method of Pregl[3], and the carbohydrates were measured by the picrate method of Willaman and Davidson.[4]

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[3] Pregl, F. Quantitative organic microanalysis. Ed. 2 English, translated from the 3rd rev. and enl. German Ed. by E. Fyleman. 237 pp. J. and A. Churchill, (London) 1930.

[4] Willaman, J. J., and F. R. Davidson. Some modification of the picric acid method for sugars. Jour. Agr. Res. [U. S.] 28: 479–488. 1924.

The Daily Rate of Growth at 85° F. and 70° F.

A significant difference has appeared, in all the experiments, between growth made at 70° F. and that made at 85° F., when measurements have been made over a period of 20 to 24 days. There can be no doubt about the net result over such periods, but if the average increase in length is plotted day by day (Fig. 2), a short initial period may appear when growth takes place faster at 85° F. than at 70° F. This fact confirms earlier observations that for a few days after inoculation, plants growing at 85° F. often appeared slightly more vigorous than those at the cooler temperature. The superiority of the growth rate at 70° F. was not apparent until about 10 days after inoculation, but was thereafter persistent. Healthy plants showed a higher growth rate at 70° F. from the start.

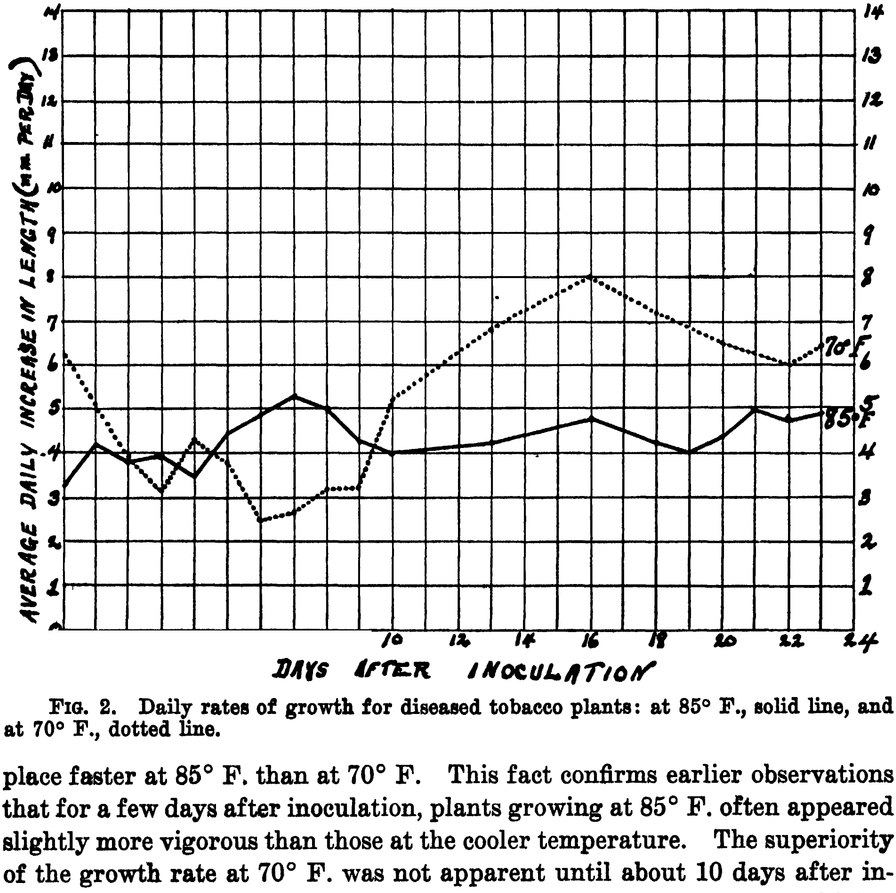

FIG. 2. Daily rates of growth for diseased tobacco plants: at 85° F., solid line, and at 70° F., dotted line.

DISCUSSION AND SUMMARY

Additional evidence of the low-temperature masking of symptoms of tobacco mosaic is presented. Symptom-free leaves appear when the host plant is grown at temperatures below 50° F. Johnson[5] established the fact of masking when the host is grown at temperatures over 98° F. The virus is thus capable of symptom-producing activity between these two temperatures, whilst tobacco plants of the variety Connecticut Havana are capable of growth, under Huddersfield conditions, between 32° F. and 108° F. The virus is present in masked symptomless leaves, but can apparently not induce mosaic mottling at masking temperatures.

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[5] See footnote 1.

Temperature relations of the virus in relation to its host can be seen very readily if the results of tables 1 and 5 are combined in the form of a graph (Fig. 3). The more exact data provided by tables 3 and 6 confirm the

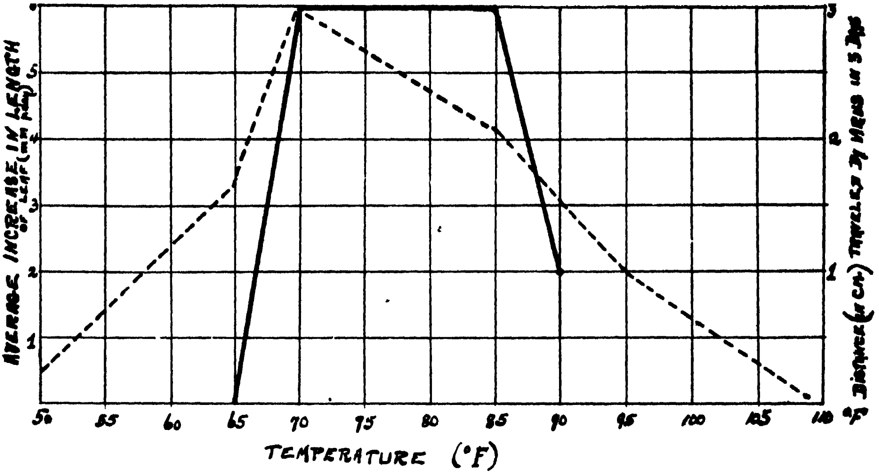

FIG. 3. Comparison of growth-rate of host (broken line) with activity of the virus (solid line) at different temperatures.

significant plateau upon the curves, namely, between 70° F. and 85° F. No difference in virus activity can be shown between these two temperatures, whilst a notable optimum of host activity appears at 70° F. The virus must therefore have a degree of independence, in that its temperature of optimal activity is not wholly coincident with that of its host.

It is most probable that other values for the optimum of host or virus activity might be found under different environments. The significant fact is that with the somewhat artificial but controlled environment used in the present experiments, the virus and its host exhibited different responses to temperature.

Daily plottings of growth rates of diseased tobacco plants at 85° and 70° F. show that for a few days after inoculation, growth at 70° may be slower than under the warmer conditions, though after 10 days the reverse is persistently true. Inversion of the curves in figure 2 between 2 days and 10 days after inoculation, coincides approximately with the spread of the virus into the young leaves round the growing point that were being measured. Virus moves at about the same rate at both temperatures; it first markedly depresses the relatively quick growth rate at 70° F., but exerts little effect upon that at 85° F. The innately higher growth rate of the host at the cooler temperature, however, is quickly reestablished. Superiority of growth of healthy plants at 70° F. is evident from the commencement of the experiment.

It is with much pleasure that the writer acknowledges the help of Mr. T. Armstrong, Head Gardener at the Ravensknowle Museum and Park, in the growth and care of plants for these experiments, and of Mrs. M. Grainger, M.Sc., for continual help with the preparation of the manuscript.

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MILD-MOSAIC VIRUS OF BROAD BEAN[1]

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INTRODUCTION

In April, 1934, plants of broad bean (*Vicia faba* L.) attacked by what appeared to be an undescribed virosis, were observed at Chang Hsing, Chekiang. Later in the same month and during May and June, further bean plants showing like symptoms were seen in many other bean-growing districts of Kiangsu Province. Studies on the properties, method of transmission, and host range of the causal virus were, therefore, undertaken. Results of the investigation are presented in this paper.

SYMPTOMS

The first symptoms appeared on the young leaves 12 to 25 days after inoculation as a fine mottle of light areas in the normal green tissue and as an indistinctly visible clearing of the veins with only slight or no tendency to rugosity. These small light-green areas gradually characterize the entire leaf surface. As the disease progresses the typical symptoms develop, *i. e.*, a diffused type of mild leaf mottling, with little or no distortion; and the slight elongation of the infected leaves (Fig. 1). The mild mottling is formed by the production of light-green areas with their margins indistinctly diffused in the green host tissues. There are neither patterns nor necrosis produced. Unlike the common mosaic of broad bean (1), the present virus produces no puckering and rugosity of affected leaves. The pods are small and weak but show no characteristic mottling. In certain cases, affected plants are stunted so slightly that it usually is not noticeable. These symptoms were constant through several successive transfers in broad-bean plants.

Under field conditions, affected plants are light yellowish green with the typical diffused type of leaf mottling. Blight of stem tips, as often seen in plants infected with common broad-bean-mosaic virus, never occurs. Plants are neither stunted nor distorted.

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[1] Paper No. 49 from the Plant Pathology Laboratory, The University of Nanking, Cheng-tu, China. The investigations upon which this paper is based were supported by a grant from the China Foundation for the Promotion of Education and Culture.

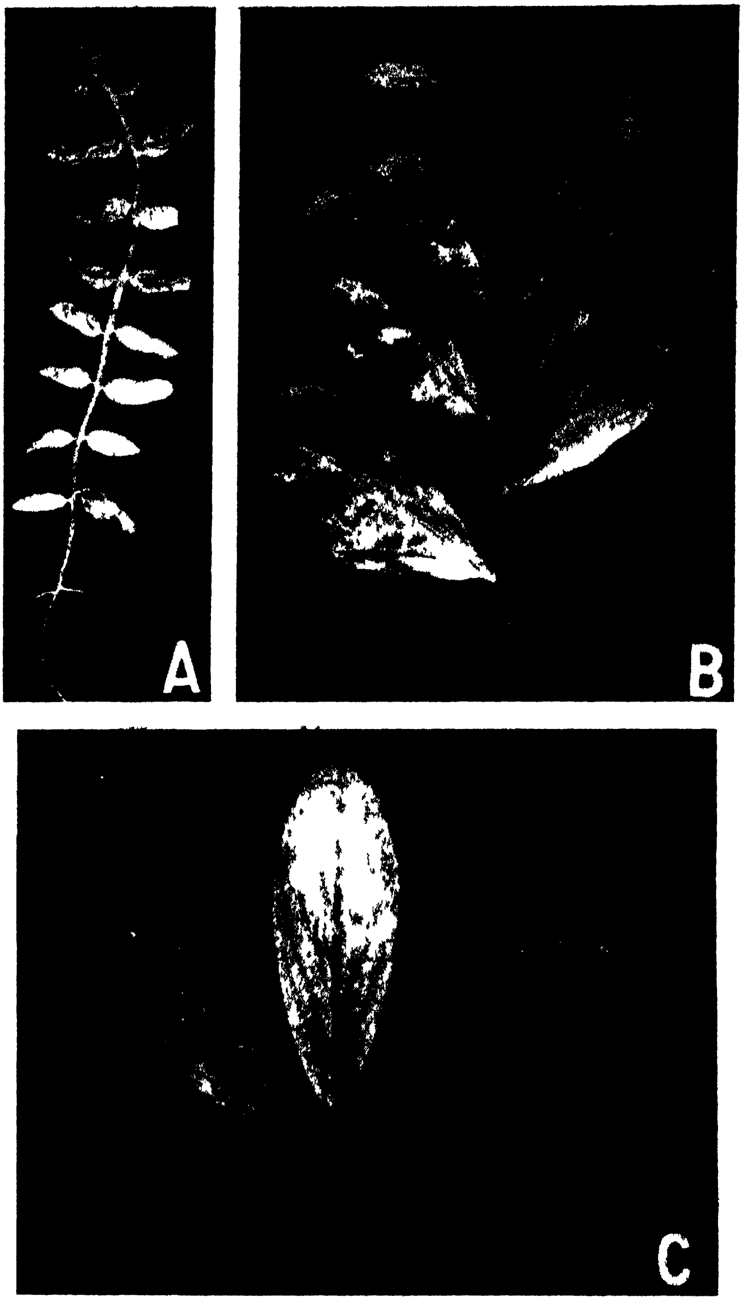

FIG. 1. Symptoms of mild mosaic. A. On spring vetch showing mottling and curling of leaves. B. On broad bean. C. On white sweet clover.

MATERIALS AND METHODS

Mild-mosaic virus of broad bean was obtained originally from naturally infected broad-bean plants growing in a field near Nanking, Kiangsu Province. The plants were transplanted from the field to a greenhouse. Several passages to seedling broad bean, spring vetch (*Vicia sativa* L.), and white sweet clover (*Melilotus alba* Ders.) by means of bean aphids (*Aphis rumicis* L.) revealed the fact that the virus was not mixed with the viruses of common broad-bean mosaic (1) and the sweet-pea mosaic, which are not infrequently found in broad bean under field conditions. The sources of inoculum were made available at all times by successive transfers from diseased broad-bean plants to vetches and red clover by *Aphis rumicis* and from broad bean to broad bean, both by aphid and by infective juice with carborundum as an abrasive.

Virus-free colonies of the bean aphid, consisting of winged and wingless viviparous females were maintained on healthy, young broad beans and hyacinth beans (*Dolichus lablab* L.) in large wire-screen insect cages. The aphids were allowed to feed on either broad-bean or red-clover plants infected with mild-mosaic virus. Later, they were transferred to healthy plants under cages. One to as many as 50 aphids were placed on each plant and allowed to remain for 2 days. Then the cages were removed and the aphids killed. The inoculum for artificial juice inoculation was prepared by grinding up mosaic-infected plants in a mortar. The expressed juice was then strained through a piece of cheesecloth and to this filtrate was added a small amount of carborundum powder (15). The small pad saturated in the inoculum was applied to the upper surface of young leaves with brisk rubbing. Inoculations were made immediately after the juice was prepared, as it was found that the virus lost its virulence rapidly outside of the host plants. Experiments were carried on in the greenhouse with an average air temperature of 20° to 24° C.

TRANSMISSION

Aphid Transmission. Mild-mosaic virus of broad bean is transmissible from diseased to healthy plants by the bean aphid. With this insect as vector, inoculations of 976 seedling broad beans, throughout 3 years 30 to as high as 60 per cent of infection was obtained. The incubation period varied from 12 to 25 days. An air temperature above 25° C. seemed unfavorable for the development of the symptoms.

The time required for the aphids to acquire the infective capacity was determined. Adult aphids, fed on healthy broad-bean plants as stock colonies, were transferred to mosaic plants under screen cages. They were allowed to remain for a desired length of time on the diseased plants and were then transferred by means of a brush to healthy seedling broad beans, where they remained for 2 days. Only the adult aphids, 25 in number, were transferred to each plant. As shown in table 1, the shortest time for them to acquire infective capacity was 10 minutes. In order to bring about a high percentage of infected plants, it was found necessary to let them feed on the mosaic plants for at least 1 hour or more.

TABLE 1.—*Time required for Aphis rumicis to acquire infectivity following transfer to mosaic broad-bean plants*

Time on mosaic plants	Percentage infected plants in experiment No.				
	1	2	3	4	5
0 (Control)	0	0	0	0	0
10 minutes	0	0	2	0	2
30 minutes	36[a]	18	42	38	16
1 hr.	44	24	54	38	52
12 hr.	42	48	40	56	16
24 hr.	52	14	58	74	36

[a] Total of 50 plants inoculated.

In determining the infectivity of aphids in relation to their stages of development, adult and nymph aphids that had been on the mosaic broad-bean plants were transferred separately to healthy plants by means of brush. Two days later the aphids were killed and cages removed. It is evident (Table 2) that both the adult and nymph aphids are of equal infective capacity.

Attempts have been made to transmit the mild mosaic virus of broad bean by other species of aphid than *Aphis rumicis.* The vectors tested were pea aphid (*Macrosiphum pisi* Kalt.), potato aphid (*M. gei* Kock.) and peach aphid (*Myzus persica* Sulz.).

TABLE 2.—*Infectivity of aphids in various stages of development*

Development stages of aphid and number employed	Percentage infected plants in experiment No.		
	1	2	3
25 wingless adult	44[a]	36	20
25 old nymph (body bright)	38	18	26
25 young nymph (body not bright)	36	24	32

[a] Total of 50 plants inoculated.

Negative results were obtained with peach and potato aphids, although *Macrosiphum pisi* transmitted the mild mosaic virus from broad bean to broad bean, vetch, white sweet clover, red clover, sweet pea, and garden pea. Their capacity for infection is, however, much inferior to that of bean aphid. Studies on aphid transmission of the virus are still in progress.

Juice Transmission. Juice transmission of mild-mosaic virus from mosaic to healthy broad-bean plants was carried on in a greenhouse by the carborundum-rubbing method. The virus was thus transmitted from broad bean to broad bean, spring vetch, winter vetch, red clover, white sweet clover, sweet pea, and *Vicia tetrasperma* Moench.

In juice-inoculated broad-bean plants, the incubation period of the virus varied from 10 to 24 days. The resulting symptoms were similar to those induced by insect vectors. Juice transmission is, however, much less successful than aphid transmission.

HOST RANGES

Studies have demonstrated that many legume viruses may infect a large number of plants besides their natural hosts. Böning (1) showed that the virus of broad-bean mosaic was infectious to pea, crimson clover and red clover. Merkel (8) succeeded in cross-inoculating many legumes with legume-mosaic viruses and came to the conclusion that the same virus is responsible for the mosaic symptoms of *Phaseolus vulgaris, Pisum sativum, Lathyrus odoratus, Melilotus altissima, Trifolium pratense, T. hybridum, T. repens, Anthyllis vulneranie* and *Vicia fabae*. Recently, Pierce (13, 14), Fajardo (3), Osborn (10, 11, 12), Zaumeyer (20, 21), Zaumeyer and Wade (22, 23, 24, 25) and Stubbs (16), all found that the legume viruses are infectious to many leguminous plants other than their natural hosts.

The following host plants were infected with mild-mosaic virus of broad bean by using the bean aphis as vector: Alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratense* L.), white clover (*Trifolium repens* L.), white sweet clover (*Melilotus alba* Desr.), spring vetch (*Vicia sativa* L.), winter vetch (*Vicia villosa* Roth.), *Vicia tetrasperma* Moench, sweet pea (*Lathyrus odoratus* L.), and field pea (*Pisum sativum* L. var. *arvense* Poir.). The virus was transmitted mechanically from broad bean to red clover, white sweet clover, spring vetch, winter vetch, *Vicia tetrasperma*, and sweet pea.

By either insect or juice transmission, the virus is not transmissible from broad bean to tobacco (*Nicotiana tabacum* L.), tomato (*Lycospersicum esculentum* Mill.), cumumber (*Cucumis sativus* L.), mung bean (*Phaseolus aureus* Roxb.), hyacinth bean (*Dolichos lablab* L.), soybean (*Sojo max* (Piper) Merr.), cowpea (*Vigna sinensis* Endl.) and kidney bean (*Phaseolus vulgaris* L.).

Symptoms in alfalfa, clovers, and peas have been observed from 14 to 27 days after inoculation by colonies of bean aphids. There were mild- and diffused-type mottlings on the leaves (Fig. 2). Affected leaves showed no rugosity, crinkling, or rolling, nor did they as in case of broad bean, show any tendency to elongate. In vetches (*Vicia sativum, V. villosa* and *V. tetrasperma*), the first symptoms of the disease usually appear in from 8 to 18 days, though a longer period sometimes is required. Symptoms in these plants are similar to those on the broad bean excepting rolling and rugosity of the lower leaflets (Fig. 3). Stunting and distortion of the plants occurred in but few instances.

CHARACTERS OF THE VIRUS

Inactivation by Dilution. Expressed juice from diseased broad-bean plants was diluted to various concentrations and inoculated immediately to seedling broad beans by the carborundum-rubbing method. The results as presented in table 3 indicates that the virus is inactivated at 1 to 2,000 dilution.

TABLE 3.—*The effect of dilution upon infectivity of broad-bean mild-mosaic virus*

Dilutions	Percentage infected plants on experiment No.		
	1	2	3
Check	36	44	28
1: 10	38	16	26
1: 100	18	32	0
1: 1,000	4	0	2
1: 1,500	0	0	2
1: 2,000	0	0	0
1: 5,000	0	0	0
1: 10,000	0	0	0

Resistance to Aging in Vitro. Five cc. of expressed juice from mosaic broad-bean plants were held for a required period in a laboratory at temperature of 20° to 22° C. The juice was then tested for infectivity by mechanical inoculation to broad-bean plants. The results (Table 4) show that the virus is rather short-lived outside of the host plants and was inactivated in less than 3 hours *in vitro.*

TABLE 4.—*Longevity of broad-bean mild-mosaic virus in vitro*

Experiment No.	Time aged									
	Fresh	5 min.	10 min.	15 min.	30 min.	1 hr.	2 hr.	3 hr.	12 hr.	1 day
1	18[a]	12	0	10	4	0	2	0	0	0
2	12	6	8	0	0	2	0	0	0	0

[a] Per cent of infected plants.

Thermal Inactivation. The thermal inactivation of the virus was tested by immersing tubes containing 2 cc. of freshly expressed leaf juice from mosaic broad bean in a water bath at the desired temperature for 10 minutes. Infectiveness of the juice was then tested by inoculating broad-bean seedlings. It is shown (Table 5) that the mild-mosaic virus was inactivated at temperatures 55° to 60° C.

TABLE 5.—*Thermal inactivation of mild-mosaic virus of broad bean*

Experiment No.	Temperatures C.				
	Control	50°	52°	55°	60°
	Percentage of plants infected				
1	28	8	2	4	0
2	16	4	0	0	0

SEED TRANSMISSION

Whether the virus of broad bean is transmitted through seed is still a moot question. Out of 9180 broad-bean plants grown from seed of mosaic-infected plants, Böning (1) obtained only 0.2 per cent of infection. Fukushi (4), working with the same virus, reported no seed transmission. Experiments conducted by the writer in the winters of 1935 and 1936, respectively, led to the same conclusion. As far as the writer is aware there is no authentic report of considerable seed transmission of viruses in broad bean.

In 1935 and 1936, seed from mild-mosaic-infected broad-bean plants, obtained from a field, was planted in a greenhouse that was fumigated at regular intervals to prevent any secondary spread by aphids. Out of a total of 2464 plants none showed mild-mosaic symptoms. It appears that the virus under investigation is not transmissible by seeds.

IDENTITY OF THE VIRUS

Mild-mosaic virus is different from the common broad-bean mosaic (1, 8) in that the latter causes, in addition to mottling, rugosity and rolling of the leaves, as well as stunting of the plants. The broad-bean local-lesion virus reported by Pierce (14) is entirely different from the viruses here considered, both as to symptomatology and host range. Broad bean, artificially inoculated, is, however, susceptible to the following additional viruses: Spotted-wilt of tomato and lettuce (5, 6, 17); common-mosaic of garden pea (7, 9); severe-mosaic of garden pea (7); red-clover mosaic (2); pea-mosaic (9, 10, 23, 24); red-clover vein-mosaic (12); lucerne virus 2 (13); ring-spot (13); speckle-pea-mosaic; marble-pea-mosaic; mild-pea-mosaic (16); celery virus 1 (18, 19); pea-streak (20); white-sweet-clover-mosaic (23, 25); alfalfa-mosaic (23, 25); and clover-mosaic (23, 25).

In comparing the symptoms and host ranges of the above-mentioned viruses, it is evident that none of them seemed to be identical with the mild-mosaic virus herein described. For the sake of clarity the present virus is designated as the mild-mosaic virus of broad bean.

SUMMARY

A mosaic disease of broad bean, caused by a hitherto undescribed virus, is here reported.

The disease here described and designated as the mild-mosaic virus of broad bean, occurs in the bean-growing regions of Kiangsu and Chekiang Province, and is not so prevalent as the common mosaic.

Broad-bean mild-mosaic is characterized by mild, diffuse type of mottling and slight elongation of affected leaves. There are no vein-banding, necrotic spotting, curvature of midrib, crinkling or rolling of leaves. Plants are neither stunted nor distorted.

Only leguminous hosts are known. The virus is infectious to broad bean (*Vicia faba,* natural host), alfalfa, red clover, white clover, white sweet

clover, spring vetch, winter vetch, *V. tetrasperma,* sweet pea, field pea, but not infectious to tobacco, tomato, cucumber, mung bean, hyacinth bean, soybean, cowpea, and kidney bean.

The insect vectors are the bean aphid (*Aphis rumicis*) and pea aphid (*Macrosiphum pisi*). Peach aphid (*Myzus persica*) and potato aphid (*Macrosiphum gei*) do not transmit the virus. It also is transmissible by artificial juice inoculations when carborundum is used as an abrasive.

The virus withstands aging *in vitro* for 3 hours at 22°–24° C. Its inactivation temperature is approximately 55°–60° C., while tolerance for dilution is about 1:1,500.

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A CHLOROTIC MOTTLING OF WHEAT LEAVES CAUSED BY INFECTIONS OF BUNT TILLETIA LAEVIS[1]

C. O. JOHNSTON AND C. L. LEFEBVRE[2]

(Accepted for publication January 18, 1939)

During the course of greenhouse experiments on the effect of bunt infection on the reaction of wheat plants to leaf rust, it was observed that bunted plants almost invariably exhibited a yellowish mottling of the leaves. The discoloration consisted of the development of light green to yellow streaks and blotches with indistinct outlines and different intensities giving the leaves a mottled rather than a distinctly spotted appearance. Typical mottled leaves are shown in figure 1.

The mottling usually appeared in the seedling stage of plant growth and persisted until maturity, often increasing in intensity as the age of the plant increased. The basal leaves usually were more severely mottled than the upper ones, especially on plants nearing maturity. Flag leaves often were only indistinctly mottled, while lower leaves of the same plant were severely discolored. In some instances the mottling did not appear on the earliest leaves but only on later ones, before the heads emerged from the boot.

In greenhouse experiments conducted in 1935 and 1936, mottling of bunt-infected plants was observed in the spring-wheat varieties, Prelude, Webster, Warden, Pusa No. 4, and a selection from the cross Pusa No. 52 × Federation. It has been seen also in bunt-infected plants of the winter wheat varieties, Turkey, Tenmarq, and Blackhull, growing in the greenhouse and in various winter varieties in the field bunt nursery.

During the greenhouse season of 1936, plants of the spring-wheat varieties, Pusa No. 4, Prelude, and Pusa 52 × Federation selection, were grown to

TABLE 1.—*The occurrence of leaf mottling in bunt-infected plants of 3 spring wheat varieties grown in the greenhouse at Manhattan, Kans., in 1936*

Variety	C. I. No.	Seed inoculated or not inoculated	Number of plants		
			Observed	Bunted	With mottled leaves
Pusa No. 4	8899	Not inoculated	50	0	0
		Inoculated	48	7	7
Prelude	4323	Not inoculated	53	0	4[a]
		Inoculated	50	25	24
Pusa 52 × Federation	11764	Not inoculated	50	0	0
		Inoculated	50	5	5

[a] 3 plants had loose smut, 2 of these had mottled leaves.

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[1] Cooperative investigations of the Division of Cereal Crops and Diseases and the Kansas Agricultural Experiment Station. Contribution No. 384, Department of Botany and Plant Pathology, Kansas State College.

[2] Associate Pathologist, Division of Cereal Crops and Diseases, and Associate Pathologist, formerly of the Kansas Agricultural Experiment Station and now with the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

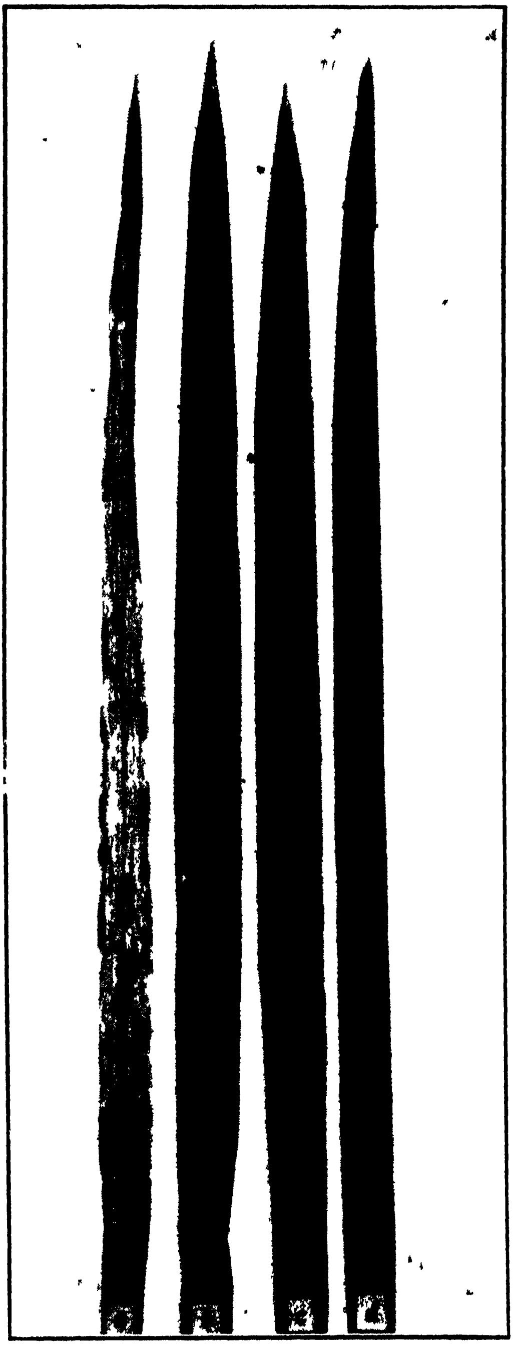

FIG. 1. Chlorotic mottling of leaves of an adult bunt-infected plant of Prelude spring wheat (*a*, *b*, and *c*) and a normal green leaf from a noninfected plant (*d*). *a*. Second leaf below the flag leaf. *b*. First leaf below the flag leaf. *c*. Flag leaf. *d*. Normal flag leaf from a noninfected plant.

maturity in 5-inch pots. The seed for approximately half of the plants was heavily inoculated with spores of *Tilletia laevis* Kühn before planting. The remaining plants came from noninoculated seed. Owing to unfavorable environmental conditions in the greenhouse, bunt infection in the inoculated series was low (Table 1). No mottling of the leaves was observed in any of the plants of the noninoculated Pusa No. 4 and Pusa 52 × Federation selection. Among the Prelude plants 4 in the noninoculated series had mottled leaves. Two of these were infected with loose smut, arising from natural inoculation. The 2 remaining plants of Prelude that exhibited chlorotic mottling of the leaves were the only mottled, non-bunt-infected plants observed during the 2 greenhouse seasons. All noninfected plants in the inoculated series were free from leaf mottling. On the other hand, table 1 shows that all of the bunted plants of the 3 varieties, except one plant of Prelude, had mottled leaves. Sterilized soil was used in all the greenhouse experiments.

Under field conditions the leaf mottling caused by bunt infection is not so easily identified as it is in the greenhouse. Competition and lack of spacing in drilled rows tend to obscure mottled plants. Certain other chlorotic disturbances, attributable to malnutrition, other diseases, and insect attacks, also make it difficult to identify mottling caused by bunt infection. In spaced sowings, however, infected plants can be identified with considerable accuracy by the presence of mottled leaves. The susceptibility of many hybrid lines of winter wheat growing in the bunt nursery in 1936 was predicted on the basis of amount of leaf mottling while the plants were in the rosette stage of growth. At heading time the earlier classifications proved to be fairly accurate. The classifications were particularly accurate for the highly bunt-susceptible and the highly bunt-resistant hybrid families, while the families of intermediate reaction were found to be most difficult to classify correctly. Many mottled plants that were tagged in the early stages of growth proved to be infected at heading time. Owing to the complicating factors mentioned, however, some plants with mottled leaves proved not to be bunted. It seems, therefore, that the presence of many plants with mottled leaves in rows grown from inoculated seed may be an indication of susceptibility to bunt, but that method of identification alone should not be considered a conclusive measure of the amount of infection.

PHYTOPATHOLOGICAL NOTES

Proposed Additions to Etiological Terminology.—The terms (1) pathogen, (2) pathogenic, and (3) pathogenicity are generally accepted to mean respectively (1) an organism capable of inducing (or inciting) disease, (2) capable of inducing disease, and (3) the ability to induce disease. The following terms are proposed for comparable concepts where decay, rather than disease, is involved. They are proposed with particular reference to the decay of sound wood, whether in a living or a dead tree or in wood products, although wider applications suggest themselves, as, for example, the decay of leaves and other organic matter in soil.

(1) *Saprogen:* an organism capable of producing decay.
(2) *Saprogenic:* capable of producing decay.
(3) *Saprogenicity:* the ability to produce decay.

The term saprogenic is recognized in Webster's New International Dictionary, second edition.

The only justification for technical terminology lies in its greater accuracy of expression and thereby its facilitation of clear thinking. On this ground these terms are proposed at the risk of overburdening an already burdensome terminology. The writer has used them in his classes in forest pathology at Idaho and found that they satisfactorily fill a gap in etiological terminology.—JOHN EHRLICH, School of Forestry, University of Idaho, Moscow, Idaho

A List of Plant Viroses Observed in China.—During the past 6 years (1932–37), surveys of important plant diseases were made by the members of the laboratory of plant pathology, Nanking University, Nanking, in various parts of China. In compiling the records of these surveys so far obtained by the writer, he has found many interesting notes on viroses. Since this group of plant diseases in China is so little known to us, a list of them is, therefore, prepared with the hope that it may be of some use to those who are interested.

ALFALFA, *Medicago sativa* L. **Mosaic.** Found in The Rural Leader's School, Nanking (Kiangsu[1]).

BEAN, *Phaseolus vulgaris* L. **Mosaic.** Very common in the gardens in Nanking and Hangchow (Chekiang), but appeared to cause no serious injury.

CABBAGE, *Brassica* spp. **Mosaic.** Found in Nanking, Hangchow, and Tsing Yang (Anhwei) and appeared to be increasing in importance in certain fields near Nanking.

CELERY, *Apium graveolens* L. **Mosaic.** As much as 20 per cent of the plants were infected with mosaic in certain fields in Wei-hsian and Tsing-chow (Shangtung) and a trace in Chengtu (Szechwan).

CHINESE PRIMROSE, *Primula sinensis* Lindl. **Mosaic.** Typical mottling was found on primrose plants in the University Garden, Nanking.

CLOVER, *Trifolium* spp. **Mosaic.** Present in the different varieties of clover on the farm of The Rural Leader's School, Nanking.

CORN, *Zea mays* L. **Mosaic.** Present on field corn in Tao Yueng (Hunan). Less than one per cent of the plants were infected. Streak also was observed in the same locality.

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[1] Name of province.

COWPEA, *Vigna sinensis* Endl. **Mosaic.** Observed in most of the cowpea-growing districts, infecting as many as 5 to 12 per cent of the plants, but with only a small reduction in yield. It was more or less prevalent in Nanking, Hangchow, Nanhsuchow (Anhwei) and Kiukiang (Kiangse).

CUCUMBER, *Cucumis sativus* L. **Mosaic.** Found in the gardens in Nanking, Suchow (Kiangsu), Hangchow, Yu-yao (Chekiang) and Nan-feng (Kiangse). The damage produced by it was very slight.

DAHLIA, *Dahlia* spp. **Mosaic.** Several Dahlia plants affected with mosaic were seen at the garden of National Central University, Nanking.

DATURA, *Datura medel* L. **Mosaic.** Found in the University Garden, Nanking.

FIG, *Ficus carica* L. **Mosaic.** Fairly general in figs growing in the University orchard. The damage was slight.

HORSE BEAN, *Vicia faba* L. **Mosaic.** Occurred in most of the bean-growing regions of Kiangsu, Chekiang, Anhwei, Kiangsi, Hupeh, Hunan and Szechwan provinces. It was very severe in Kiangsu especially in Western part of province. In Nanking and its vicinity, infection was from a trace to 100 per cent. Heavy losses are due to blight of stem tips and stunting of plants.

Rosette. It was first seen in the spring of 1935 at a field of the university farm. The disease was very common in Nanking and vicinity. The characteristic symptoms are rosetting and yellowing. Affected plants may be little more than a compact tuft of small, chlorotic, curled, and distorted leaves. It produced from 5 to 50 per cent loss to the crop and is certainly one of the important diseases of bean plantations. The disease is hitherto unreported.

Mild mosaic. Only 1 to 5 per cent plants infected with the disease in certain fields in Nanking. Disease not serious.

HYACINTH BEAN, *Dolichus lablab* L. **Mosaic.** Found in Nanking and Hangchow. The disease was of little importance.

LETTUCE, *Lactuca sativa* L. **Mosaic.** General in Kiangsu, Chekiang, Hupeh and Szechwan provinces; but the damage was only slight.

LIMA BEAN, *Phaseolus limensis* Macf. **Mosaic.** About 2 per cent of mosaic was present in the university garden, Nanking.

NASTURTIUM, *Tropaeolum majus* L. **Mosaic.** Found on Nasturtium in a greenhouse, Chengtu (Szechwan).

PEA, *Pisum sativum* L. **Mosaic.** Both the mild and severe types were very prevalent in Southwestern part of Shangtung and Northern part of Kiangsu provinces. It was very severe in some cases in Tai Hsing and the surrounding districts (Kiangsu).

Streak. Found in combination with mosaic in certain pea fields in Nanking.

PEANUT, *Arachis hypogeae* L. **Mosaic.** Present in many fields from Yen-chow to Taian (Shangtung). In some fields infection was abundant; in others slight. Mottling and leaf deformity were pronounced. In general, less than 1 per cent of mosaic plants were stunted.

PEPPER, *Capsicum annuum* L. **Mosaic.** Very widely distributed throughout the pepper-growing district over the whole country; but not a serious factor in pepper production.

PETUNIA, *Petunia violacea* Lindl. **Mosaic.** Several mosaic-affected plants were found in the university garden.

POTATO, *Solanum tuberosum* L. **Leaf roll.** General in potato plantations of Kiangsu province. Of 174 fields examined, 5 to 31 per cent of the plants were affected. At the farm of National Bureau of Agricultural Research, Nanking, leaf roll was found on Irish Cobbler, Bliss Triumph, and Green Mountain varieties.

Mosaic. General throughout the potato sections of the provinces; severe in certain fields.

Spindle tuber. Observed in fields of Bureau of Agriculture, Nanking. It was not widely prevalent.

Witches'-broom. Seen on Green Mountain and Burbank potatoes in the university garden. The disease only sparsely prevalent.

PUMPKIN, *Cucurbita pepo.* **Mosaic.** Present in the university horticultural garden, Nanking; caused no noticeable damage.

RADISH, *Raphanus sativus* L. **Mosaic.** Found on different varieties of radishes in a greenhouse, Horticulture Department, Nanking University, Nanking.

RAPE, *Brassica campestris* L. var. Chinese oil rape. **Mosaic.** Widespread in Kiangsu and Chekiang provinces; of little importance.

RASPBERRY, *Rubus* spp. **Mosaic.** Observed on raspberry in horticultural garden, Nanking University; caused no noticeable damage.

SOYBEAN, *Glycine max* Merr. **Mosaic.** General in all important soybean-growing regions of the country. In Kiangsu, it was present in 64 out of 317 fields inspected. In

Anhwei, the average infection was 0.3 to 1 per cent. Beans were affected 100 per cent with mosaic in certain fields along Kao-tsi railway in Shangtung province. It caused heavy damage to beans in these districts.

SPINACH, *Spinacia oleracea* Mill. **Mosaic.** Prevalent quite generally in Nanking and vicinity. Infections ranged from less than 1 per cent to as much as 50 per cent. The reduction of yield was, however, not noticeable. The disease was found also in Chekiang, Anhwei, Kiangsi, Hupeh and Hunan provinces.

SQUASH, *Cucurbita* spp. **Mosaic.** Present each year in the gardens near Nanking. Damage was not noticeable.

SUGAR CANE, *Saccharum officinarum* L. **Mosaic.** Common in Fukien; reported as a serious disease of cane in Szechwan province.

SWEET POTATO, *Ipomoea batatas* Lam. **Mosaic.** Found on sweet potatoes in a greenhouse, Chekiang University, Chekiang.

TOBACCO, *Nicotiana tabacum* L. **Mosaic.** Mild mosaic was commonest in the important tobacco-growing regions of Shangtung and Hsen Ning (Hepeh). It was severe when plants were affected at the early stages; development resulting in stunting of the plants.

Ring spot. Fairly common in Hsen Ning and certain tobacco-growing sections in Szechwan. In certain fields, 100 per cent of plants were infected. Not seen in Shangtung.

Spot necrosis. Observed in Hsen Ning.

Leaf curl. Seen in Hsen Ning.

TOMATO, *Lycopersicum esculentum.* **Mosaic.** General in gardens of Kiangsu, Chekiang, Honan, and Shangtung provinces. Infections from 5 to as much as 100 per cent.

Streak. General in Nanking; caused considerable loss in some plantations.

Leaf curl. Fairly common in Kiangsu, Chekiang, Honan, and Shangtung provinces. Tomatoes severely damaged by the disease.

Rugose mosaic. Seen in Nanking.

Leaf roll. Severe in Nanking.

WHITE CLOVER, *Trifolium repens* L. **Mosaic.** Found in Nanking.

WHITE SWEET CLOVER, *Melilotus alba* Desr. **Mosaic.** Present on university farm, Nanking.

—T. F. YU, *Plant Pathology Laboratory, Nanking University, Cheng-tu, Szechwan, China*

ANNOUNCEMENT

The annual summer meeting of the Pacific Division of the American Phytopathological Society will be held on the Stanford University campus, Palo Alto, California, June 27–30, 1939. Presentation of papers and discussion of research problems will occupy four half days. In addition a symposium on the teaching of plant pathology will be held. One day has been reserved for a pathological field trip through the extensive ornamental and truck crop producing areas south of San Francisco. Accommodations for guests will be provided in the University dormitories. Pathologists from all sections are invited to attend and to participate in the meetings. A complete program will be furnished upon request.—L. D. Leach, Secretary-Treasurer. Pacific Division of American Phytopathological Society, Davis, California.

THE HEMLOCK RUST CAUSED BY MELAMPSORA FARLOWII

GEORGE H. HEPTING AND E. RICHARD TOOLE

(Accepted for publication Feb. 20, 1939)

INTRODUCTION

In 1935 reports were received of extensive damage to eastern hemlock (*Tsuga canadensis* (L.) Carr) and of lesser damage to Carolina hemlock (*T. caroliniana* Engelm) resulting from the blighting of the current season's shoots. These reports came from commercial nurseries in western North Carolina growing hemlocks for ornamentals. The disease has been present for many years and has been consistently causing considerable financial loss largely through disfigured and, hence, unsalable trees. The cause of the twig blight was found to be *Melampsora farlowii* (Arth.) Davis, an autoecious rust not previously reported as of any economic importance (6). The rust attacks leaves and cones, as well as twigs. The present investigation was conducted to determine the life history of the rust in the North Carolina mountains and means of control in nurseries. The work was done in 1938 in commercial nurseries from Pineola to Blowing Rock, North Carolina. All information reported, unless otherwise stated, refers to the disease as it occurs in this section.

SYMPTOMS

The first symptom of the disease in the spring is a yellowing of some of the new leaves about one month after the buds burst, which in western North

FIG. 1. The top of a young eastern hemlock showing twig blight caused by *Melampsora farlowii*. Note the drooping or curling of most of the diseased twigs. × $\frac{1}{12}$.

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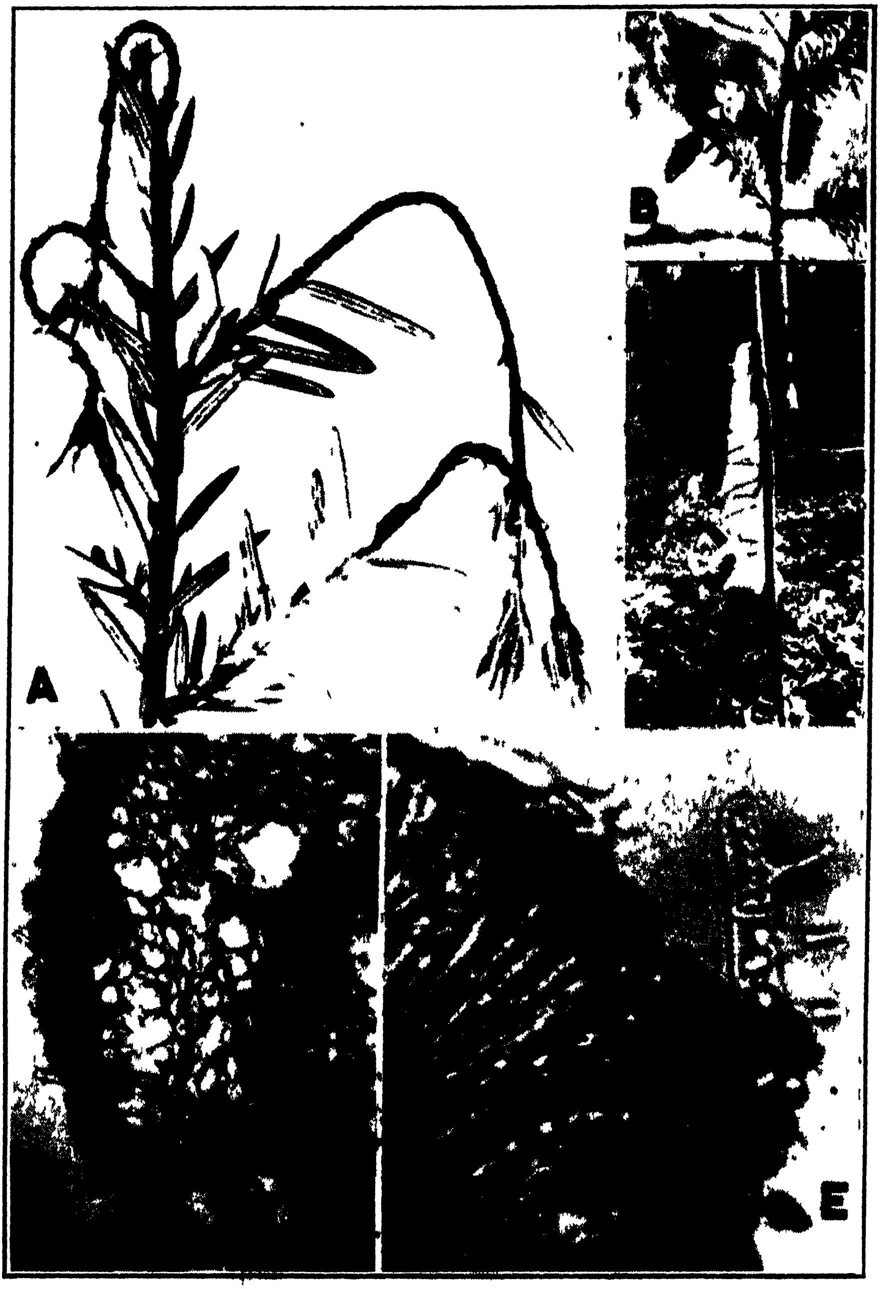

FIG. 2. A. Shoots of eastern hemlock infected with *Melampsora farlowii*. ×2. Telia had formed in the dark-colored drooped parts of the twigs. Photographed on July 21, 1938. B. Upper cone normal; lower cone infected with *M. farlowii*. ×½. C. Top of a small hemlock placed in a York chamber attached to stake for inoculation. ×1/14. D. Cross section of a hemlock twig showing telia. ×100. E. Teliospores and basidium of *M. farlowii*. Only 3 of the 4 sterigmata are evident on the basidium ×700.

Carolina is roughly the end of May. Within a week or 10 days after the leaves turn yellow those parts of the new shoots about the bases of some of the yellowed needles turn orange-colored and later become flaccid, often causing the shoots to droop (Fig. 1 and 2, A). Most of the infected needles soon drop off. As the season progresses many of the infected shoots curl and lose all their needles from the region of infection to the tips, giving them the characteristic appearance shown in figure 1. The dead shoots usually remain in place a year or more, causing the trees to appear as though they had been singed around the outside. Within a week or two after infection has become evident the subepidermal telia of the fungus can frequently be found on the leaves and always on the shoots (Fig. 2, D and E).

Infected cones remain closed, do not produce seed, and are frequently discolored, shrivelled, and mummified. Figure 2, B, shows a normal open cone and one infected and unopened. Small swollen places on the cone scales indicate the presence of telia.

RANGE AND ECONOMIC IMPORTANCE

Melampsora farlowii is widely distributed in eastern United States and Canada. Fraser (4, 5) reported it on eastern hemlock in Nova Scotia, Arthur (2) in a number of eastern States and Wisconsin, Hunt (8) in three New England States, and the authors have found it in the mountains from Virginia to northern Georgia. Ordinarily, this rust apparenty does little damage, killing only occasional twigs and aborting cones. While it can be found commonly in natural stands along the Appalachian range, the attack is usually so light as to be inconspicuous. The only report of severe damage by this disease prior to that of Hepting and Davidson (6) on the North Carolina nursery infections was by Fraser (5), who stated that the rust did considerable injury to young hemlocks near Pictou, Nova Scotia, in 1910 and 1911.

Nursery-grown hemlocks between about 2 and 15 feet high are especially liable to severe attack in the section between Pineola and Blowing Rock, North Carolina. Hemlock hedges in this section have also been severely damaged almost annually by the rust for at least the past several years. In the case of nursery-grown trees and hedges especially good conditions prevail for the extraordinary development of the disease because the trimming of the trees and their proximity to each other result in extensive areas of compact foliage. Repeated attacks dwarf trees and sometimes result in their death. Even fairly light attacks often make them unsightly and greatly reduce their value for commercial purposes. Table 1 gives the percentage of shoots killed in representative areas at 3 nurseries and in a natural stand of hemlock adjacent to one of them. The trees sampled were from 2 to 5 feet high. The methods of sampling are given in the footnote to the table. The heaviest attack was in the upper third of the trees and on the primary shoots. The field in which 82 per cent of the 1938 shoots were killed was rendered practically worthless as a result of being severely attacked for several seasons. Table 1 indicates an increase in incidence of the rust in 1938 over 1937 in most fields. Part of this increase, however, is probably due to the breaking off of some of the shoots killed in 1937 without leaving any evidence by the time these counts were made in 1938. Table 1 also indicates that the susceptibility of eastern hemlock is higher than that of Carolina hemlock.

Infection is not limited to small trees or the lower parts of large trees. Abundant twig and cone infection was found in the tops of two eastern hemlocks, 80 and 45 feet high, growing near one of the nurseries. The following percentages of cones were diseased at different heights on the 80-foot tree: at 5 feet, 81 per cent; 18 feet, 85 per cent; 30 feet, 58 per cent; 40 feet, 59 per cent; 50 feet, 62 per cent; 60 feet, 62 per cent; and 70 feet, 36 per cent. Forty-five per cent of the cones on the 45-foot tree and 41 per cent of those on a hedge 5 feet high were diseased. Since diseased cones produce no seed, these figures show that the rust can materially affect the seed crop.

TABLE 1.—*Percentage of hemlock shoots infected by Melampsora farlowii on representative nursery areas*

Nursery		Species	Twigs infected[a]	
			1937	1938
			Per cent	*Per cent*
A	Field 1	*Tsuga canadensis*	41	82
	Field 2	*Tsuga canadensis*	18	45
	Field 3	*Tsuga canadensis*	20	37
	Field 4	*Tsuga caroliniana*	6	19
	Field 5	*Tsuga caroliniana*	11	1
	Natural stand on edge of A	*Tsuga canadensis*	27	25
B		*Tsuga canadensis*	25	
C		*Tsuga canadensis*	42	

[a] The percentages for nursery A were obtained in the following manner: In each area 10 trees, 2–5 feet high, were selected at random. Each of these trees was divided into three equal height zones. Within each of these zones 50 shoots were selected on randomly chosen branches and classified into infected and uninfected. The percentages for nurseries B and C were based on a different number of shoots in each zone, selected at random.

ETIOLOGY

Melampsora farlowii (Arth.) Davis was first described by Arthur (1) under the name *Necium farlowii* Arth. Saccardo later placed the fungus in the genus *Chrysomyxa,* and in 1915 Davis (3) transferred it to *Melampsora.* No pycnia, aecia, or uredia have been found. The telia are waxy and orange-colored when fresh and are composed of single palisades of teliospores that form just beneath and within the epidermis. Measurements were made of 10 teliospores from each of 10 twigs, and the average dimensions were 55 × 9 μ. The average diameter of 50 germinating sporidia was 8 μ. These dimensions are within the range given by Arthur (2) and Fraser (5) for this rust. The sporidia are spherical and reddish-yellow.

Inoculations

In 1912 Fraser reported successful inoculations of *Tsuga canadensis* with sporidia of *Melampsora farlowii* in the laboratory (4). He found the first indication of infection 9 days after inoculation and telia after 16 days. In the present study inoculations were made at Pineola, North Carolina, on nursery transplants of both *T. canadensis* and *T. caroliniana* during the 1938 growing season. The sources of inoculum were twigs killed by the rust in 1937. These were collected and placed in Petri dish moist-chambers until the teliospores produced abundant sporidia. This usually took from 24 to 48 hours. Prior to inoculation trees to be inoculated were sprayed with water by means of an atomizer. Six twigs with germinating teliospores were then fastened above each tree, after which the trees were again sprayed with water and covered by inoculation chambers. Two types of chambers, the so-called iceless refrigerator (7) and the chamber described by York (11), were used. The type of iceless refrigerator was the modification of Snell and Gravatt (10) and consisted of three parts—a reservoir, a support, and a cloth covering. The York chamber consisted of a celluloid cone covered with moist cotton batting (Fig. 2, C). The latter proved the more satisfactory in the preliminary work and was used in all subsequent inoculations. Each inoculated tree was paired with a control tree similarly treated with the exception that no inoculum was suspended over it. After 24 to 40 hours the chambers and inoculum were removed.

On May 4 the tops of two 3-foot-high eastern hemlocks were placed under York chambers Inoculum was suspended over one, and the other served as a check. Infection became evident May 19 in the inoculated tree. At this time no natural infection was evident in the nursery. On June 6, 40 per cent of the 198 shoots in the chamber that had contained inoculum were infected and none of the 172 checks. A similar pair of trees was set up on May 19. Infection was evident on May 31. On June 6, 68 per cent of the inoculated shoots were infected and 6 per cent of the checks. Table 2 gives the results

TABLE 2.—*Results of inoculation of hemlock seedlings with Melampsora farlowii*[a]

Inoculation date	Tree species	Inoculated trees		Controls	
		Trees used	Trees infected	Trees used	Trees infected
		Number	*Number*	*Number*	*Number*
May 25, 1938	*T. caroliniana*	4	3	4	0
June 15, 1938	*T. caroliniana*	2	2	2	0
June 16, 1938	*T. canadensis*	4	4	4	0
June 17, 1938	*T. canadensis*	4	2	4	0
June 22, 1938	*T. canadensis*	4	2	4	0
June 23, 1938	*T. canadensis*	4	2	4	0
All		22	15	22	0

[a] Trees from 5 to 18 inches tall. Inoculations made in York chambers. Six twigs bearing germinating teliospores suspended above each inoculated tree.

of inoculations on a series of trees 5 to 18 inches high. On these trees infection was evident after an average incubation period of 14 days. In most cases the inoculated trees which did not become infected had ceased new growth before the time of inoculation and had no young foliage.

Life History

The sources of inoculum in the spring are the telia in the overwintered twigs and cones killed the preceding spring. The germination of overwintered teliospores was tested in moist-chambers from early April until the middle of July. Germination was very slight until the latter part of April, shortly before the hemlock buds burst. The period of most active growth of the hemlock shoots was from early May until the middle of June. Sporidia were produced abundantly in moist-chambers from early May through the first week of June. Thus, shoot growth of the hemlock and sporidial production by the rust occur almost concurrently. By the middle of June sporidial production had practically ceased. Teliospores started germinating naturally in the field after a few days of rain in early May.

Teliospores germinate in place, forming large promycelia on which reddish-yellow sporidia are formed (Fig. 2, E). These sporidia infect the current season's leaves, which turn yellow about 14 days after infection. From the leaves infection apparently spreads into the shoots where telia develop just beneath the epidermis. Telia are also produced in the leaves, which shed soon after infection. Telia can be found within 2 or 3 weeks after infection, but they do not germinate until the following spring. A very poor seed crop precluded following the course of the disease on cones.

EPIDEMIOLOGY

The incidence of this disease has been observed to be affected by the amount of inoculum present, rainfall, the condition of the new growth at the time sporidia are produced, and apparently altitude or temperature. The density of the foliage and the large numbers of trees close together in nurseries favor the extensive development of the disease. Field observations indicate that periods of 10 or more hours of rainy weather are necessary before teliospores will germinate. The duration of the rain seems more important than the amount. Thus, short spring showers often do not last long enough for sporidia to be produced. Dew is not sufficient for teliospore germination.

Trees in poor vigor or recently transplanted are often late in starting shoot growth. At one nursery the buds on several unthrifty trees did not burst until 2 weeks or more after the more thrifty trees. The opportunities for infection of such trees are thus greatly reduced.

The severity of the disease seems to vary with altitude. It may be that the lower temperatures at the higher altitudes favor development of the fungus. The nurseries that have been attacked severely are at elevations between about 3500 and 4000 feet. At another nursery, about 2500 feet in elevation and only a few miles from one of the heavily infected nurseries, the hemlock has been almost free from the rust. Since the rust occurs commonly in northeastern United States and Canada it is reasonable to expect that its optimum development in the South would be at the higher elevations.

CONTROL

Hemlock trees grown for ornamentals have a relatively high value. They may remain in transplant rows 10 or more years, thus representing a considerable investment. Attempt at direct control of the twig rust, such as by spraying or dusting, is warranted under these conditions, while it would not be in the forest. To attempt to exclude the pathogene from nurseries by removing and burning all infected twigs and cones before growth starts in the spring would be costly and difficult to do with sufficient thoroughness. The application of protectants seemed the most feasible control measure to try.

In preliminary trials sprays were found easier to apply and were more efficient in coverage per unit volume of chemical than dusts. Even gentle breezes caused a large proportion of the dust to be wasted. Dusts were therefore tentatively eliminated and the season's work confined to two spray materials: Bordeaux mixture and lime-sulphur. The composition of the Bordeaux was 4–4–50 (4 lb. hydrated lime) and of the lime-sulphur, 4 lb. of the powdered form to 50 gals. of water. To both spray solutions casein spreader[1] was added at a rate of 2 lb. to 50 gal. of solution.

The randomized block split plot type of experimental design was used. Five blocks were laid out in representative parts of the hemlock fields in a nursery at Pineola, North Carolina. Each block consisted of 16 eastern hemlocks, 2 to 5 feet high, that were part of the experiment, with other trees scattered among them to serve as sources of inoculum, eliminating border effect. The blocks were split in half at right angles to the rows, with 8 trees in the Bordeaux series and 8 in the lime-sulphur. One of each series served as a check, and each of the other 7 was sprayed according to one of the following schedules: weekly or every two weeks until the end of May, until the end of June, until July 11, and before rains. All spraying was begun with the bursting of the majority of the buds on May 2. There was no replication within blocks. The trees to be sprayed according to the different schedules were selected at random. Damage counts were made on July 25, according to the first method described in the footnote to table 1.

In addition to the experimental blocks successive rows at two locations in the nursery were sprayed weekly with Bordeaux and casein spreader, Bordeaux and raw linseed oil,[2] and lime-sulphur and casein spreader. These sprays were prepared at the same strengths as for the blocks. A check row was left in each series. Damage counts were made on July 22.

RESULTS

The percentages of shoots infected for the different spray materials and schedules in the different blocks are shown in table 3. A marked difference

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[1] Purchased from Grasselli Chemical Co.

[2] Linseed oil added at a rate of 5 qts. to 50 gals. of prepared Bordeaux and emulsified by forcing the mixture through a spray gun (9).

TABLE 3.—*Percentage of eastern hemlock shoots infected by Melampsora farlowii in sprayed blocks*

Spray and block number	Infections on trees sprayed[a]							Unsprayed
	Through May		Through June		Through July 11		Rains Before	
	Weekly	Bi-weekly[b]	Weekly	Bi-weekly	Weekly	Bi-weekly		
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
Bordeaux								
Number 1	6	12	14	7	5	9	8	21
Number 2	11	17	6	12	6	11	10	21
Number 3	4	7	6	7	7	8	15	21
Number 4	19	0	9	21	13	3	13	30
Number 5	7	12	7	0	1	7	3	9
All blocks	9	10	8	9	6	8	10	20
Lime-sulphur								
Number 1	1	5	1	6	3	7	9	12
Number 2	8	15	5	9	8	15	8	17
Number 3	1	7	5	2	7	4	11	28
Number 4	4	11	4	15	7	5	7	25
Number 5	0	2	1	6	3	4	6	7
All blocks	3	8	3	8	6	7	8	18

[a] All spraying was begun on May 2, when the majority of the buds had burst. The sampling method used to estimate the percentage infection is that described in the first part of the footnote to table 1.

[b] Every two weeks.

is evident between the percentage of diseased shoots on the checks and on the sprayed trees. The differences between spray materials and among schedules are not so marked, although, lime-sulphur appears superior to Bordeaux. These data were subjected to the analysis of variance, and the results are given in table 4. There were significant differences among blocks, between treatment and no treatment, and between spray materials. Further analysis revealed no significant differences among the spray periods; that is, spraying

TABLE 4.—*The analysis of variance in percentage of hemlock shoots infected by Melampsora farlowii in sprayed blocks*

Source of variation	Degrees of freedom	Sum of squares	Mean square	Odds that the factor is significant
Whole blocks				
Block	4	503.18	125.80	> 20: 1
Spray material	1	135.20	135.20	> 20: 1
Block-spray interaction	4	40.67	10.17	
Sub-total	9	679.05		
Half blocks				
Treatment	7	1325.95	189.42	> 100: 1
Spray-treatment interaction	7	81.60	11.66	< 20: 1
Remainder	56	1156.95	20.66	
Sub-total	70	2564.50		
Total	**79**	**3243.55**		

through May was just as effective as through June or the middle of July. Spraying weekly with lime-sulphur gave significantly better control than biweekly. The most efficient results were obtained by spraying weekly with lime-sulphur only through May. The critical infection period will vary from year to year with weather conditions. Thus, if the month of May were very dry, sporidial production might take place mostly in June and, therefore, June would be the critical month to spray. Spraying before rains is not a very satisfactory method in the North Carolina mountains because showers are often of daily occurrence, and it is frequently difficult to determine whether or not it will rain or for how long. The results of spraying before rains were equivalent to spraying biweekly.

In addition to their protective effect on the foliage the sprays were effective in preventing the production of sporidia from overwintered telia. The teliospores in shoots sprayed with either material failed to germinate in Petri dishes and were not observed germinating on the trees. This probably accounts in a large measure for the check trees having an average of only 19 per cent of the shoots infected, whereas the eastern hemlocks at certain other parts of the nursery (Table 1) had from 37 to 82 per cent of their 1938 shoots infected. The checks were surrounded by trees most of which were producing no inoculum. This sanitary effect indicates that the control to be expected from spraying all hemlocks in a field will probably be much superior to that obtained in the experimental blocks where unsprayed trees were capable of producing abundant inoculum.

The average number of infected shoots per tree in the sprayed rows were: lime-sulphur with casein spreader 5, Bordeaux with linseed oil 6, Bordeaux with casein spreader 10, and checks 25. Here, again, the number of infections on the checks was low compared with those on unsprayed trees in other parts of the nursery. In spite of precautions some spray undoubtedly fell upon the check trees since they were adjacent to sprayed rows. This condition also obtained in the blocks.

RECOMMENDATIONS AND COSTS

The control obtained by spraying with lime-sulphur weekly through May was sufficient to keep the stock in good condition. Until the most efficient schedules can be worked out for the different weather conditions, it is suggested that all hemlocks in affected nurseries be sprayed with lime-sulphur at the time the buds burst and once a week for the following 4 weeks. This suggested schedule is by no means fixed since it is possible that fewer sprayings in May will be just as effective where all hemlocks are sprayed, and it is probable that some additional spraying should be done in June if May is very dry.

The cost of spraying will vary with the size of trees, the number of applications, and the type of equipment used. Records kept during the experimental spraying will serve to give an indication of costs. The sprayed trees ranged from about 2 to 5 feet in height, and a 4-gallon hand sprayer was used. Allowing 25 cents per hour for labor, the average cost per tree per application was 3 mills for labor and 1 mill for chemicals (Bordeaux or lime-sulphur) and depreciation of equipment, giving a total cost of 4 mills per tree per application. If 5 applications were made per year the cost would be 2 cents per tree per year. Where power equipment is used and large-scale spraying is done this unit cost can probably be reduced.

SUMMARY

A twig blight of hemlock, proved by inoculations to be caused by the autoecious rust *Melampsora farlowii* (Arth.) Davis, has been causing considerable financial loss annually in some commercial nurseries in western North Carolina. This rust, which also attacks leaves and cones, is widely distributed in eastern United States and Canada but apparently does severe damage only under conditions of dense hemlock foliage, such as in nursery-grown trees and in hedges.

Eastern hemlocks in localized areas in nurseries in western North Carolina had as high as 82 per cent of their 1938 shoots killed by the rust. The amount of infection varied between and within nurseries. Carolina hemlock is much more resistant than eastern hemlock.

The sources of inoculum in the spring are the telia on the overwintered twigs and cones killed the preceding spring. In western North Carolina sporidia are usually produced from early May until the middle of June, during the period of most active growth of the hemlock shoots. Twig infection apparently takes place through the needles. At least 10 hours of rainy weather seem to be necessary for sporidial production.

In experiments to control this rust at one nursery Bordeaux mixture, 4-4-50 (hydrated lime), and lime-sulphur (4 lb. of the dry form to 50 gal. of water) were used, following several spray schedules. The most efficient control was obtained by spraying with lime-sulphur weekly through May. Trees so treated had 3 per cent of their twigs killed, compared with 19 per cent on unsprayed trees in the experiment. The cost of spraying was 4 mills per tree (2 to 5 feet high) per application.

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GENETIC AND ENVIRONMENTAL FACTORS AFFECTING GROWTH TYPES OF USTILAGO ZEAE[1]

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This paper presents the results of a study on the relative importance of genetic and environmental factors in determining growth types of haploid lines of the corn-smut fungus, *Ustilago zeae* (Beckm.) Ung., on artificial media.

Hanna (3), Christensen (2), and Stakman, Christensen, *et al.* (9) called attention to the fact that cultures of some monosporidial lines of *Ustilago zeae* consisted almost entirely of mycelium, others almost entirely of sporidia, and still others of varying mixtures of the two. Stakman (7) reported a 2 : 2 segregation for growth type in the monosporidial lines derived from a single promycelium. Similar evidence of segregation for growth type has been reported by Rodenhiser for other smuts (4, 5).

In connection with an extensive investigation of the genetics of *Ustilago zeae*, it was observed that the 3 general growth types mentioned above were common among monosporidial lines. It was evident that growth type was determined, to some extent at least, by genetic factors and that segregation of factors took place in the promycelium. However, the behavior of some lines was puzzling. All such lines had been derived from single sporidia that multiplied for a time by budding. Some lines remained sporidial throughout the entire period of growth on artificial media; colonies of other lines quickly and uniformly became mycelial; and the colonies of still others remained sporidial for a time and then became mycelial in patches or in sectors that appeared at different times and in different places in the same colony. Because the change from sporidial to mycelial type took place at different times, the appearance of the mycelial patches or sectors was not always the same. For this reason it was difficult or impossible to determine by visual inspection whether variants were being produced or whether there was merely an irregular change to a mycelial growth type; and it was necessary to make transfers from all of the mycelial sectors or patches to ascertain whether they resulted merely from a transformation of growth type or from genetic variation.

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[2] The author wishes to express his sincere thanks to Dr. E. C. Stakman for his helpful suggestions and criticisms during the study and for help in the preparation of the manuscript.

For the above reasons a study was made of the factors affecting the sporidial and mycelial types of growth. Inasmuch as some lines seemed to be predominantly sporidial, others predominantly mycelial, and still others intermediate, a study was first made of the effect of environmental factors on the growth type.

EXPERIMENTAL PROCEDURE AND RESULTS

Effect of Environmental Factors on Growth Types

In studying the cultures it was necessary to begin with lines that were relatively constant for their particular growth types. In order to select suitable lines, many monosporidial lines were obtained from E. C. Stakman. These lines were grown on potato-dextrose agar in Erlenmeyer flasks and were examined macroscopically and microscopically for growth type. From them were selected 4 mycelial lines, 2 sporidial lines, and 7 intermediate lines that produced different proportions of sporidia and mycelium in the colonies.

The designation of the monosporidial lines indicates their origin. For example, $10A_4$ is a monosporidial line isolated from the basal or fourth cell of the promycelium of chlamydospore A resulting from cross 10.

In studying the effects of various nutrients on the growth types of *Ustilago zeae*, a synthetic nutrient solution similar to that recommended by Brown (1) was used in hanging-drop cultures. The solution contained 20 g. of dextrose, 2.5 g. of asparagin, 2.5 g. of tribasic potassium phosphate, 0.2 g. of magnesium sulphate, and 1000 cc. of water. The concentration of each nutrient was varied within the following ranges: dextrose, 0.2 to 20 per cent; asparagin, 0 to 1.5 per cent; magnesium sulphate, 0 to 0.5 per cent; and tribasic potassium phosphate, 0 to 1.0 per cent. When the concentration of a single nutrient was varied, all of the others were held constant in the proportions designated in the basic medium.

Each solution was sterilized by autoclaving for 20 minutes at 15 pounds' pressure. Drops of the solution to be used were placed on the lower surface of sterile cover slips on van Tieghem cells in Petri dishes, six cells in each dish. Moistened filter paper was placed in the bottom of the dishes to prevent drying of the drops, and the cells were placed in holes cut through the filter papers to facilitate microscopic examinations of the cultures.

Monosporidial lines $10A_4$ (mycelial), $18C_3$ (sporidial), and $22B_2$ (intermediate) were used throughout the experiments, and each culture was replicated 12 times. After inoculation, the cultures were examined at intervals of one to several hours until the growth types appeared stable.

Effect of Dextrose and Sucrose

Stakman (6) showed that certain cereal smut-fungi produce predominantly sporidia on the promycelium when the chlamydospores germinate in sugar solutions but have a tendency to produce hyphal branches and only few sporidia in water. The writer, therefore, experimented to determine whether or not different concentrations of dextrose and sucrose in nutrient media would change the growth types of sporidial, mycelial, and intermediate smut lines. The effects of the following concentrations of these sugars were compared: 0.2 per cent, 1 per cent, 10 per cent, and 20 per cent.

The results with dextrose and sucrose were practically identical. The type of growth of sporidial line $18C_3$ was the same in all of the solutions. The mycelial line $10A_4$ produced a few sporidia in all of the solutions immediately after inoculation, the largest number being produced in the more concentrated solutions. In the weaker solutions the mycelial line ceased to produce sporidia within about 20 hours, while in the more concentrated solutions it continued producing them for about 48 hours. The rate of growth likewise was much more rapid in the more concentrated solutions. There were great differences in the response of the intermediate line $22B_2$ to the different concentrations of these sugars. In fresh solutions containing 1 per cent or more of sugar the intermediate line became completely sporidial, and, like the mycelial line, produced more sporidia in the more concentrated solutions. In the weakest solution the intermediate line became mycelial after about 32 hours, while the cultures in the most concentrated solution did not change from sporidial to mycelial growth until after approximately 80 hours. Likewise, as with the mycelial line, the rate of growth increased as the concentrations of the sugars were increased.

These results show clearly that increasing the concentration of dextrose and sucrose in the nutrient medium greatly increases the tendency of intermediate lines to produce sporidia, but only slightly increases the tendency of mycelial lines to produce sporidia. The rate of growth of these lines also increased in the more concentrated solutions, indicating some association between rate of growth and number of sporidia produced.

Comparative Effect of Various Sugars

Since the concentration of dextrose and sucrose in nutrient media greatly influenced the growth type of some smut lines, the question immediately arose as to what effect other sugars would have on these lines. Accordingly, experiments were made to test effects of mannose, maltose, levulose, lactose, raffinose, rhamnose, and xylose. In this experiment a 20 per cent concentration of each sugar was used, the solutions containing dextrose and sucrose serving as controls.

The sporidial line did not produce mycelium in any of the solutions, but the mycelial line produced varying numbers of sporidia, depending on the particular sugar. In the solutions containing dextrose, mannose, levulose, sucrose, raffinose, and xylose the mycelial line ceased producing sporidia after 52 to 58 hours and became entirely mycelial; in the solution containing lactose it produced no sporidia after 32 hours, while in the solution containing rhamnose it remained strictly mycelial. The intermediate line behaved very much like the mycelial line in these solutions, except that a far larger number of sporidia were produced. The results of these experiments also indicated clearly that the amount of sporidial growth in mycelial and intermediate lines is associated with the supply of easily available carbohydrates. For example, rhamnose did not stimulate the production of sporidia in the mycelial or intermediate lines, and none of the lines used in this experiment grew very well in the solution containing it. On the other hand, those sugars that stimulated the production of some sporidia induced a correspondingly greater amount of growth.

Effects of Asparagin

From the previous results it seemed likely that the ratio of carbohydrate to nitrogen in the nutrient solutions might have an important influence on the growth types of these smut lines. Experiments, therefore, were made with asparagin as the source of nitrogen.

In determining the effects of nitrogen on these sporidial, intermediate, and mycelial lines, asparagin was added to the basic solution in amounts sufficient to bring about concentrations of 0.02 per cent, 0.5 per cent, and 1.5 per cent. The basic solution lacking asparagin was used as the control.

Again the sporidial line remained unchanged. The mycelial line produced a few sporidia in all of the solutions immediately after inoculation. In the solutions containing 0, 0.02 per cent, and 0.5 per cent asparagin, the mycelial line ceased producing sporidia after 35, 41, and 44 hours, respectively; but, when the concentration was increased to 1 5 per cent, no sporidia were produced after 28 hours. The intermediate line followed the same general trend as the mycelial line in producing more or fewer sporidia in the different solutions, but it never became entirely sporidial or entirely mycelial in any of the solutions. These results indicate that sporidial growth in the intermediate and mycelial lines increases with an increase in the amount of asparagin, but if the amount of this nutrient is increased beyond a certain limit it becomes deleterious and reduces the proportional number of sporidia produced. In the most concentrated solution the rate and amount of growth were both considerably reduced.

Effect of Various Mineral Salts

To determine whether or not different concentrations of the mineral salts in the basic medium would affect the growth types of the sporidial, mycelial, and intermediate lines, experiments were made with magnesium sulphate and tribasic potassium phosphate in the concentrations indicated in table 1. In another set of experiments dibasic and monobasic potassium phosphate were substituted for the tribasic salt.

From table 1 it will be noted that the growth of the sporidial line was not changed in any of the solutions. It may also be noted that the intermediate and mycelial lines produced sporidia for a longer time, and, consequently, more sporidia, when a 0.02 per cent concentration of these salts

TABLE 1.—*The effect of magnesium sulphate and tribasic potassium phosphate on the growth types of a mycelial, a sporidial, and an intermediate haploid line of Ustilago zeae when grown in hanging-drop cultures*

Mineral salt	Percentage basic medium	Number of hours after inoculation after which sporidial production ceased		
		$10A_4$ (mycelial)	$22B_2$ (intermediate)	$18C_3$ (sporidial)
$MgSO_4$	.0	27	46	Constant
	.02	44	48	Do.
	.25	40	46	Do.
	.5	Constant	46	Do.
$K\ PO_4$	.0	21	64	Constant
	.02	25	70	Do.
	.25	21	64	Do.
	1.00	21	64	Do.

was used; while, at higher concentrations, the production of sporidia diminished or was entirely suppressed. When dibasic or monobasic potassium phosphate were substituted for tribasic potassium phosphate, the results were practically identical.

Effect of a Fresh Supply of Nutrients

All of the above results indicate that a fresh supply of nutrients induced the mycelial line to produce some sporidia and the intermediate line to produce more than the usual number. For this reason an intermediate line, $22B_2$, and a mycelial line $10A_4$ were transferred to fresh drops twice daily for 5 days. The intermediate line became entirely sporidial after the first 3 days, but the mycelial line produced only a few sporidia. When these cultures were allowed to grow undisturbed they soon resumed their normal growth types.

Other Factors

Since it seemed apparent that mycelial production was associated with lack of nutrients, the sporidial line $18C_3$ was grown in sterile distilled water for several days, but it showed no tendency whatever to produce mycelium, even under these conditions.

Results of an experiment in which the solidity of the medium was varied showed that the amount of sporidial growth tended to increase in proportion to mycelial growth as the percentage of agar in the medium was decreased. This was especially true of the two intermediate lines used, but very little effect was noticeable with the two mycelial lines tested.

Temperature, hydrogen-ion concentration of the medium, and the addition to potato dextrose agar of "staling" products from smut cultures had no effect on the 3 growth types.

In summarizing the results of the preceding experiments, it is clear that the use of nutrients had more effect on growth types than the other environmental factors. The effects of all the nutrients were strikingly similar but were expressed to a greater or lesser degree with particular nutrients, sucrose and dextrose having the most marked effect. The sporidial line did not change, irrespective of the medium used. Although the mycelial line became intermediate under certain conditions, the writer wishes to emphasize the fact that only few sporidia were produced under any circumstances, and ultimately the cultures again became strictly mycelial. The intermediate line responded much more to the different nutrients. It produced either all sporidia in some fresh solutions or produced a much greater proportion of sporidia than mycelium in other fresh solutions. As the cultures became older and grew more slowly, they became either strictly or predominantly mycelial, depending on the particular solution used.

In all cases, except those where excessive nutrients were used, the amount of sporidial growth in the intermediate line was directly proportional to the quantity of nutrient in the medium. This was especially pronounced when the amount of sucrose and dextrose was varied. An excess of certain nutrients apparently had a deleterious effect, as there was a reduction in the relative number of sporidia produced and in the total amount of growth. On the other hand, growth was vigorous in all solutions in which large numbers of sporidia were produced. When nutrients were withheld or exhausted, or growth was retarded because of the presence of a certain nutrient in large enough quantities to be deleterious, the intermediate smut line tended to become mycelial, and the mycelial line ceased to produce sporidia.

THE INHERITANCE OF FACTORS FOR SPORIDIAL AND MYCELIAL GROWTH TYPES

The results of the studies on the effects of environmental factors on growth types produced by a selected number of lines indicated that the intermediate lines appeared to have factors for both sporidial and mycelial growth; hence the growth types could be shifted by certain environmental factors, particularly the supply of carbohydrates.

The mycelial lines, although relatively stable, produced a few sporidia in response to certain environmental factors. The growth type of the strict sporidial lines could not be altered appreciably by such factors.

Apparently, therefore, the range of variability of sporidial lines is quite limited and that of mycelial lines fairly so. The expression of characters in such lines seems to be more dependent on genetic than on environmental factors. For this reason a study was made of the inheritance of factors for these types of growth, especially since studies by Stakman and his coworkers had indicated that factors for these two types of growth were definitely inherited.

Table 2 indicates the growth types of the monosporidial lines isolated from one of the crosses made by Stakman and his coworkers. As will be noted, there was clear-cut segregation of factors for sporidial and mycelial growth types at the time of chlamydospore germination. These haploid lines were obtained by the writer and used in further studies of the inheritance of factors for sporidial and mycelial growth types.

In the inheritance studies, a susceptible variety of corn, Northwestern Dent, was used as the host Crosses were made by mixing liquid cultures of monosporidial lines in the desired combinations and hypodermically in-

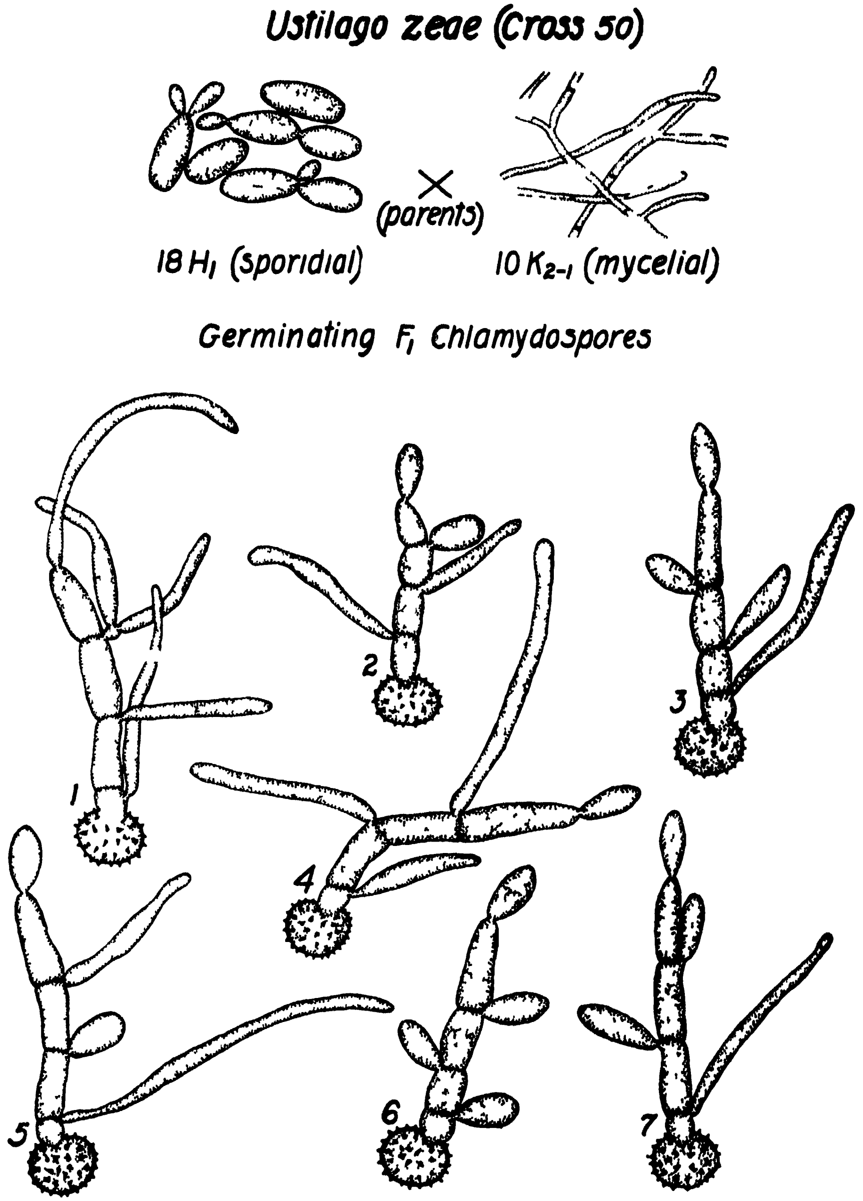

FIG. 1. The growth types of the parents of Cross 50 and germinating F_1 chlamydospores, showing the production of sporidia and hyphal branches on the promycelia in the following ratios: 1—0S: 4M, 2 and 3—2S: 2M (paired), 4—1S: 3M, 5—2S: 2M (alternating), 6—4S: 0M, 7—3S: 1M. The germinating chlamydospores were drawn free hand, approximately × 900.

TABLE 2.—*Growth types of haploid lines of Ustilago zeae isolated from Cross 18 ($10K_2 \times 101_1$), immediately after isolation*

Haploid line from promycelial cell	Chlamydospores and growth types[b]							
	A	B	C	D	E	F	G	H
No.								
1[a]	S	S	S	M	M	M	M	S
2	S	S	S	S	M	M	M	S
3	M	S	S	M	S	S	S	M
4	S	S	S	S	S	M	S	S

[a] Tip cell of the promycelium.
[b] S = sporidial; M = mycelial.

jecting the mixture into the corn plants. The method was essentially the same as that described by Stakman and Christensen (8). To make sure that none of the lines used was solo-pathogenic, each monosporidial line also was inoculated separately into the host.

Eight of the segregates of Cross 18, which had remained strictly sporidial, were crossed with several rather strict mycelial lines in order to study more carefully the inheritance of the two growth types. One of the crosses ($18H_1 \times 10K_{2-1}$) caused heavy infection, with abundant production of chlamydospores; therefore, it was selected for study and was arbitrarily designated Cross 50.

Chlamydospores of Cross 50 were germinated and it was found that sporidia and hyphal branches were produced on the promycelia in the ratios of 4: 0, 3: 1, and 2: 2, as shown in figure 1.

When 4 sporidia were produced on a promycelium they were isolated and grown in culture in the usual manner. Forty-five single sporidia were isolated from the promycelia of 13 chlamydospores of Cross 50, and the resulting monosporidial lines grown in duplicate in 250-cc. Erlenmeyer flasks containing 40 cc. of potato-dextrose agar. Table 3 indicates the growth types of the resulting monosporidial lines immediately after isolation, and figures 2 shows the growth types of the parents and 12 monosporidial lines isolated from 3 chlamydospores of the cross. That the sporidia do not

TABLE 3.—*Growth types of haploid lines of Ustilago zeae isolated from Cross 50 ($18H_1 \times 10K_{2-1}$), immediately after isolation*

Haploid line from promycelial cell	Chlamydospores and growth types[b]												
	A	B	C	D	E	F	G	H	I	J	K	L	M
No.													
1[a]	S	–	S	S	M	S	S	S	M	–	S	S	–
2	S	S	S	S	M	M	S	M	M	M	M	M	M
3	S	S	–	S	M	M	M	M	S	M	–	S	M
4	M	M	M	M	S	S	M	M	–	M	M	–	S

[a] Tip cell of the promycelium.
[b] S = sporidial; M = mycelial; – = no sporidia isolated.

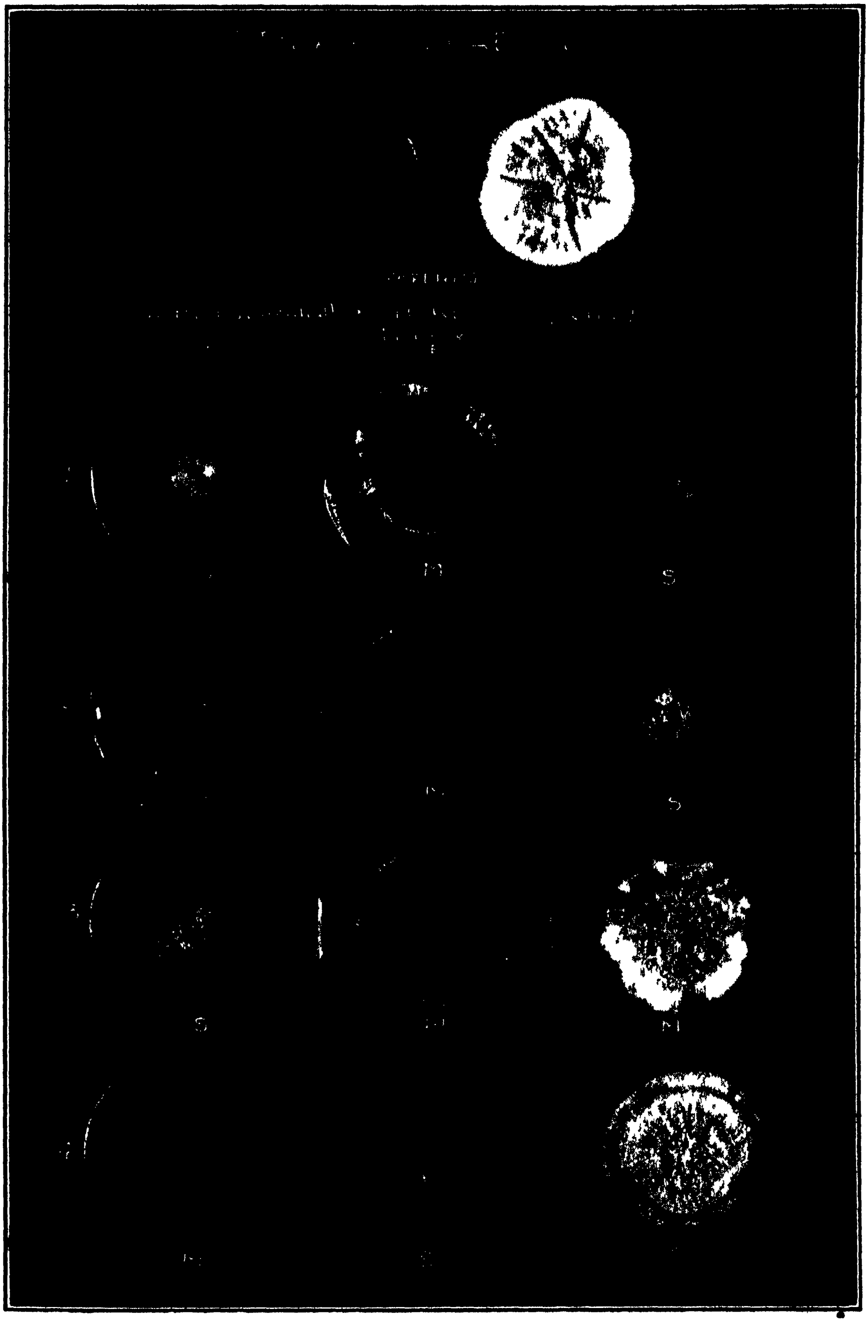

FIG. 2. The parents, $18H_1$ and $10K_{2\text{-}1}$, of Cross 50 and cultures of the 12 monosporidial lines obtained by isolating the sporidia from the promycelia of three chlamydospores, D, E, and G, resulting from the cross. In each case the cultures are numbered according to the position of the sporidia on the promycelia from which they were obtained, number 1 being from the tip cell. S = sporidial, M = mycelial. Note the segregation ratios: Chlamydospore D—3S: 1M, E—3M: 1S, G—2S: 2M.

necessarily produce sporidial colonies is evident, since some of the colonies became mycelial (Fig. 2). However, ten lines from this cross remained strictly sporidial. It is apparent from these results that monosporidial lines isolated from a single promycelium and grown in culture may subsequently form sporidial and mycelial colonies in the ratios of 4:0, 3:1, or 2:2.

The type of growth from the individual promycelial cells of 100 germinating chlamydospores from Cross 50 was observed. Of the 400 cells, 247 produced sporidia and 153 produced hyphal branches. Since most haploid lines do not form mycelial colonies until some time after isolation, one would expect more sporidia than hyphae to be produced on the promycelia.

Hyphal branches from 15 promycelia were cut off with a microrazor and cultured singly on potato-dextrose agar. The resulting colonies were always mycelial on this medium. Four of them were paired in all possible combinations among themselves and with the remaining 11 lines of the 15, and inoculated into corn seedlings. Their behavior was the same as that of haploid lines resulting from single sporidia, since lines of opposite sex caused normal infection and development of chlamydospores. Clearly then, these mycelial branches are partly gametic in nature, just as the sporidia are. Naturally, there are no special gametes in *Ustilago zeae,* as both sporidia and haploid hypal branches can multiply or grow and also behave as gametes. It is evident from the results of these experiments that there is segregation for sporidial and mycelial growth types at the time of germination of chlamydospores. It seems apparent to the writer that there are two or more factors governing these growth types and that the intermediate growth types result from various balances between factors for sporidial and mycelial growth. The results further indicate that, although lines may contain factors for intermediate and mycelial growth types, these characters are not necessarily expressed until after the lines have reached a certain age.

DISCUSSION

Prior to the present study, several investigators (2, 3, 4, 5) had observed different growth types in monosporidial lines of some of the smuts; but relatively little had been known concerning the factors that determine their development.

From the results of the writer's studies, it is clear that different growth types in *Ustilago zeac* are determined by the interaction of genetic and environmental factors, as would, of course, be expected. However, certain sporidial lines apparently have factors for sporidial development only, as they remained sporidial despite all attempts to change them by altering environmental conditions. In fact, it has not been possible to bring about infection in corn by inoculating with combinations of strict sporidial lines, possibly because these lines do not have the necessary factors for the production of mycelium. The habit of some mycelial lines is less rigidly fixed by genetic factors than that of the strict sporidial lines. Some of the mycelial lines produce sporidia in the early stages of growth and become mycelial later. As there is a difference between different lines in their tendency to change from the sporidial to the mycelial type of growth, it seems probable that several genetic factors are involved. Some of the lines resulting from the hyphal branches cut off from the promycelia seem to be strictly mycelial, as they never have produced any sporidia. Some of the other mycelial lines, however, are capable of producing sporidia under suitable environmental conditions. It seems, therefore, that there are strict sporidial lines, strict mycelial lines, mycelial lines that are capable of producing a few sporidia, and a group of intermediate lines that differ in their tendency to change from the sporidial to the mycelial type. It is evident from the inheritance studies that there is clear cut segregation on the promycelia of certain chlamydospores of factors for the production of sporidia and hyphal branches. The morphological difference is sharp and unmistakable. On culturing single sporidia, 3 types of lines can be obtained: strict sporidial, intermediate, and mycelial lines that can produce a few sporidia under certain conditions. When hyphal branches were cut off from promycelia, however, some strict mycelial lines were obtained.

The above facts suggest that these growth types are dependent on multiple factors, and there is substantial proof for this opinion. Segregation occurs in the promycelia of germinating chlamydospores, and the resulting haploid lines are gametic in nature. Consequently, if these characters were determined by a single-factor pair, there would always have to be a 1:1 segregation for them in each promycelium, but the results of these investigations show that this is not the case.

The dependence of these growth types on multiple factors may explain some of the peculiar responses to environment. If, for example, the growth type of an intermediate line is determined by a balance of factors for sporidial and mycelial growth, it seems very likely that environment could shift the growth type one way or another. On the other hand, sporidial and mycelial growth types that may result from a predominance of sporidial or mycelial factors, respectively, are not nearly so susceptible to environmental changes.

Further evidence that there are multiple factors for growth type is that some intermediate lines have a greater proportion of sporidial to mycelial growth, whereas others are predominantly mycelial, although they are definitely intermediate.

All facts considered point toward the following general conclusions: (a) that there are 2 or more factors for growth type, (b) that growth types are determined genetically, and (c) that the environment influences intermediate lines considerably but has no appreciable effect on sporidial lines and but little effect on mycelial lines.

SUMMARY

Studies were made on the relative effects of genetic and environmental factors in determining growth types of *Ustilago zeae*.

The following 3 growth types were shown to exist: (a) a strict sporidial type, (b) a rather strict mycelial type, and (c) various intergrades of intermediate types.

The strict sporidial types could not be induced to produce mycelium under any conditions of the experiments.

The rather strict mycelial type could be induced to produce only very few sporidia under certain conditions.

Some intermediate types could be induced to become either sporidial or mycelial with the proper environment.

In nutrient solutions, increasing the quantity of various nutrients, especially certain sugars, increased the sporidial growth in intermediate lines and to a much lesser extent in mycelial lines.

Fresh supplies of nutrients increased sporidial growth in intermediate lines and to a much lesser extent in rather strict mycelial lines.

Temperature, hydrogen-ion concentration of the medium, and "staling" products had no effect on the growth types of any of the lines studied.

There was segregation of factors for sporidial and mycelial growth types on individual promycelia on a 4:0, 3:1, and 2:2 basis, in a sporidial × mycelial cross.

The results indicate that there are two or more factors for both sporidial and mycelial types.

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DISTRIBUTION AND PREVALENCE OF OZONIUM ROOT ROT IN THE SHELTERBELT PLANTING AREA OF OKLAHOMA

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AND ERNEST WRIGHT[3]

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INTRODUCTION

Earlier reports in the literature indicate that the ozonium root rot fungus, *Phymatotrichum omnivorum* (Shear) Duggar, can be found in the two lower tiers of counties adjacent to the Red River in southern Oklahoma, extending as far east as Miller County, Arkansas. A preliminary survey in southwestern Oklahoma in 1935 verified the presence of root rot in certain counties within the shelterbelt planting area.

Employing the same methods as described by the senior writer[4] a detailed survey of part of this area was completed in August, 1938. The chief objective of the survey was to furnish the Prairie States Forestry Project of the U. S. Forest Service an accurate map showing the locations of infested and ozonium-free land. On the basis of the information provided, shelterbelt plantings can be made with safety, either by avoiding ozonium-infested regions or by using resistant species on infested soil.

RESULTS OF THE SURVEY

The distribution of ozonium root rot as determined by the survey is presented in figure 1. The apparent relationship between ozonium-infested areas and the watersheds, as well as the drainage basins of the larger rivers, was as striking as that observed in Texas in 1935, so that the results of the survey will again be presented on the basis of watersheds and drainage basins of the larger streams.

It will be noted from figure 1 that the western limit of root-rot infestation extended somewhat beyond the 99° meridian in Tillman and Kiowa Counties. The entire drainage basin of the North Fork of the Red River was free of root rot, except for the one limited area.

Otter Creek, with its source in the Wichita Mountains, was of especial interest because root rot did not occur north of the creek, although it was very prevalent to the south. Beyond the point where Otter Creek passes through a well-marked sand ridge and the channel becomes considerably deeper, root rot could not be found. The northern limit of root rot in this valley was located in Kiowa County in a low-lying basin subject to overflow.

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[1] Chairman, Department of Bacteriology, University of Nebraska; Agent, Bureau of Plant Industry, U. S. Department of Agriculture, from August 1 to September 10, 1938.

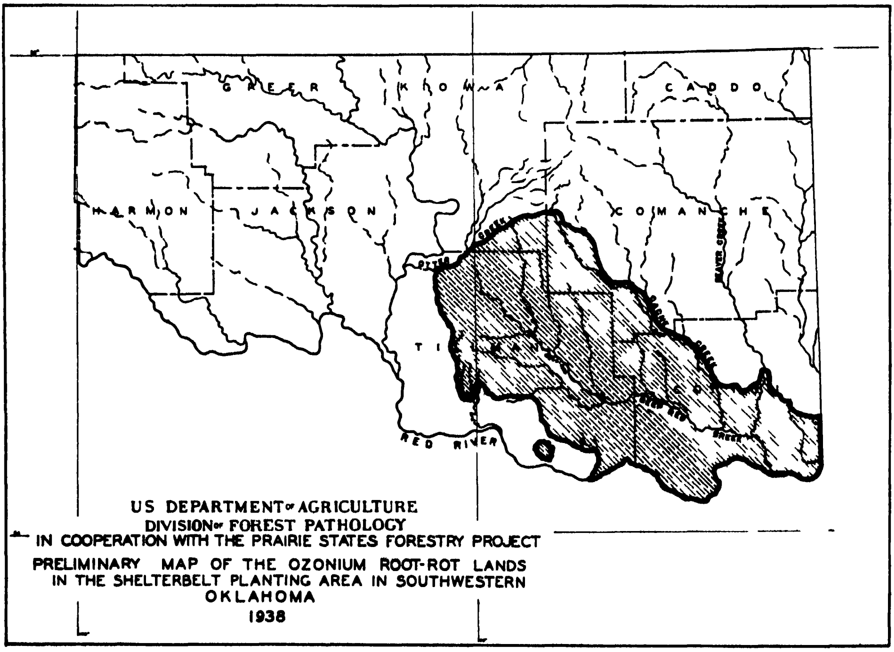
[2] Agent, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, from August 1 to September 10, 1938; formerly Assistant Pathologist.

[3] Associate Pathologist, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

[4] Peltier, G. L. The distribution and prevalence of ozonium root rot in the shelterbelt zone of Texas. Phytopath. 27: 145–158. 1937.

A like situation was found in the valley of a small creek originating near Frederick, Oklahoma. Here, again, as the creek ran from the heavier lands through a sand ridge into lighter, sandy soils, root rot was not found. Just what environmental factors had inhibited the fungus under these conditions could not be determined from field observations. Similar instances were noted in Texas in 1935.

Root rot was found in the entire drainage basin of the Deep Red Creek in Tillman County and north into southeastern Kiowa County. The prevalence of root rot in this watershed varied considerably, but was more severe in the better agricultural lands and in the valleys of the creek and its tributaries. The fact that the greater part of the infested area was planted to wheat and other nonsusceptible, fibrous-rooted crops, further attested to the heavy root-rot infestation in this watershed.


GREER
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TILLMAN
RED RIVER
US DEPARTMENT OF AGRICULTURE
DIVISION OF FOREST PATHOLOGY
IN COOPERATION WITH THE PRAIRIE STATES FORESTRY PROJECT
PRELIMINARY MAP OF THE OZONIUM ROOT-ROT LANDS
IN THE SHELTERBELT PLANTING AREA IN SOUTHWESTERN
OKLAHOMA
1938

FIG. 1. The distribution of ozonium root rot in the shelterbelt planting area of southwestern Oklahoma.

In the narrow watershed of the Red River in south Tillman County, a small isolated infested area of approximately 4 square miles was mapped. This area has been recognized for about 30 years, according to one of the landowners. By a series of posts this man had staked out the root-rot infestation on his farm. He stated that the fungus had not spread since he obtained the farm in 1906. Several miles east of this isolated area root rot was traced to the bed of the Red River.

In general, it may be stated that the western line of demarcation between noninfested and infested lands was extremely sharp, and, to a large extent, coincided with the transition between light and heavy soil types. This was not true, however, in southeastern Tillman County, where root rot was prevalent on very sandy soil. The northern limit of infestation in this district extended to the foothills of the Wichita Mountains, in southeastern Kiowa County in the headwaters of Deep Red Creek, and in southwestern Comanche County in the headwaters of the Main West Cache Creek. The only infested areas in Comanche County were in the drainage basin of the above-named creek. All other drainage basins in this county were free of root rot.

Approximately the lower two-thirds of Cotton County can be classified as infested. Of course, the prevalence of root rot varied from field to field, but, in the main, a high degree of infestation occurred in the better and more cropped lands, especially in the drainage basins of the Deep Red and West Cache Creeks. Root rot was not observed in the drainage basin of Beaver Creek and in the upper Cache Creek watershed. The lower Cache Creek basin showed a varying root-rot infestation to a point where it enters the Deep Red Creek. Root rot was mapped from Cache Creek east to the county line, the northern limit being about 3 miles south of the point where Cotton, Stephens, and Jefferson Counties adjoin. This completed the survey of those counties lying in the shelterbelt planting area.

Root-rot infestation extends eastward from this point, however, as shown by the records on file in the Department of Botany and Plant Pathology, Oklahoma Agricultural Experiment Station, which were made available to us by K Starr Chester, who also made 2 reconnaissance trips into this area in August and September, 1938. He reported (personal communication) having seen root rot in every county adjacent to the Red River from Cotton through McCurtain, and also in several of the second tier of counties to the north. He called attention also to several isolated areas well beyond the northern limit of root-rot infestation, only one, near Sayre, Oklahoma, being active at the present time. Possibly this isolated area had its origin in the introduction of infected planting stock.

In a recheck of certain surveyed areas in Texas it was of extreme interest to note that the lines of demarcation were not only as pronounced, but that no apparent extension of root rot beyond the limits mapped in 1935 had taken place. Further, a reconnaissance survey of the sandy lands suitable for tree planting in north Wichita and Clay Counties revealed that the disease was more or less continuous and of varying severity in the area between the Wichita and Red Rivers.

DISCUSSION

In figure 2 the combined root-rot-infested areas mapped during the summers of 1935 and 1938 in Texas and Oklahoma are shown. The predominant feature of these surveys was the sharp demarcation between infested and noninfested areas (Fig. 3, A). No explanation of this striking phenomenon can be offered at this time, although perhaps low temperatures (including the depth of the frost line), available soil moisture, and to some extent soil types may be contributing factors. The northward extension of root rot to the foothills of the Wichita Mountains in Oklahoma may be attributable to the protection afforded by them from cold north winds. Since the western limit of soil infestation coincides approximately with the transition between prairie (*i.e.*, mixed grasses) and the infested wood land (chiefly mesquite and shin and post oaks), there appears to be a correlation between types of natural vegetation and root rot.

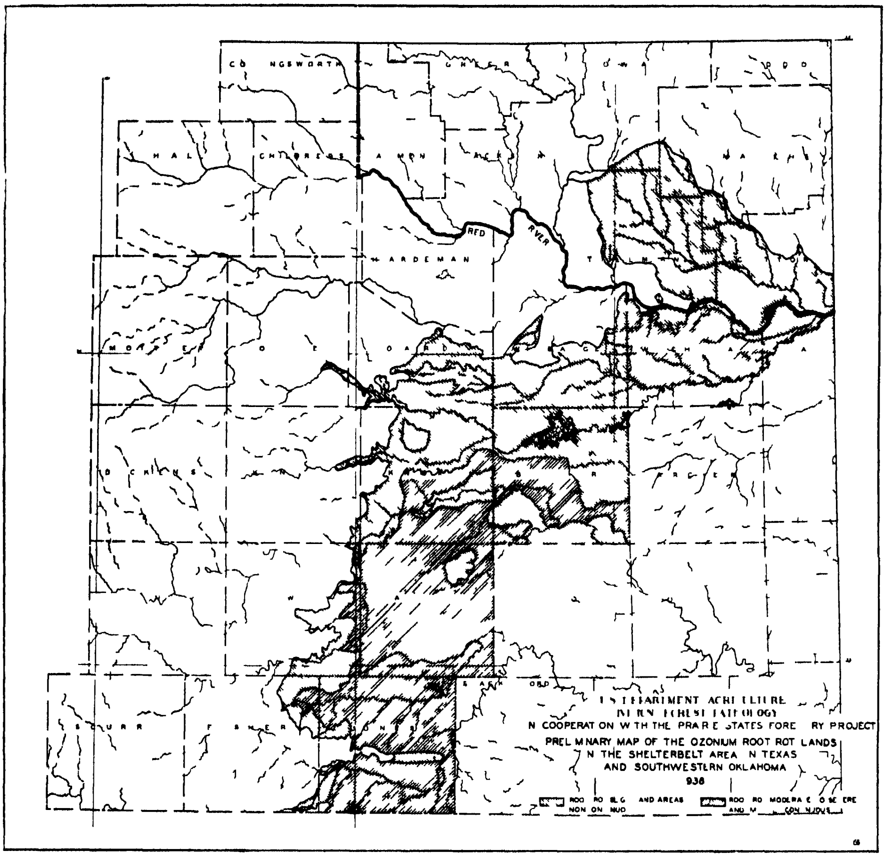
RED RIVER
HARDEMAN
U S DEPARTMENT AGRICULTURE
DIVISION FOREST PATHOLOGY
IN COOPERATION WITH THE PRAIRIE STATES FORESTRY PROJECT
PRELIMINARY MAP OF THE OZONIUM ROOT ROT LANDS
IN THE SHELTERBELT AREA IN TEXAS
AND SOUTHWESTERN OKLAHOMA
1938
ROOT ROT SLIGHT AND AREAS NONCONTINUOUS
ROOT ROT MODERATE TO SEVERE AND M CONTINUOUS

FIG. 2. The distribution and prevalence of ozonium root rot in the shelterbelt planting area of southwestern Oklahoma and Texas.

A second outstanding feature was the apparent cumulative incidence of root rot from the headwaters of a creek down the drainage basin. This situation was clearly evident in the better agricultural lands in continuous cultivation to susceptible crops. A good illustration is the drainage basin of Deep Red Creek in Oklahoma.

Lastly, the futility of clearing native ozonium-infested lands for diversified farming was likewise apparent during 1938 in both Oklahoma and Texas. In one instance approximately 5600 acres, for a resettlement project, was opened for cultivation and irrigation in 1936. Already 50 per cent or more of the alfalfa plantings are gone, together with a loss of 90 per cent of the current cotton crop in some fields (Fig. 3, B). The planning of suitable rotations for soil conservation projects is handicapped by the presence of root rot, especially where orchards, shelterbelts, and soil-building crops are included, unless this factor is taken into consideration. In 1937 and 1938 several experimental shelterbelts were planted on infested sites. Root rot already has taken its toll of susceptible tree species, including Chinese and American elms, cottonwoods, and locusts (honey and black). It is evident, therefore, that surveys for ozonium root rot should be made before undertaking such projects, and, if the areas are found infested, they can be either avoided or planted to resistant crops.

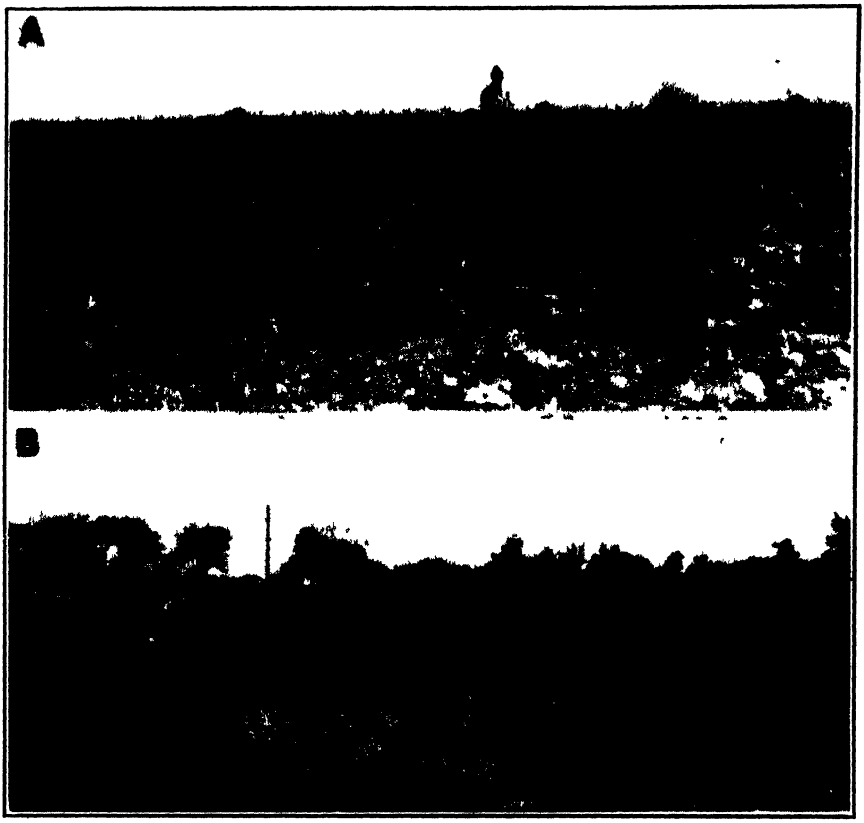

FIG 3 A View in cotton field showing line of demarcation between ozonium-infested part on the left and non infested area on the right (Adams Creek Watershed near Vernon, Texas). B. A large ozonium root rot spot in a 2 year old alfalfa field under irrigation near Wichita Falls, Texas.

SUMMARY

By methods previously employed, certain areas in southwestern Oklahoma were surveyed and mapped, showing the locations of ozonium-infested land. The necessity of such surveys is apparent, since they provide a basis whereby shelterbelt plantings can be made either in non-infested areas or on infested lands with resistant tree species.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
IN COOPERATION WITH
FOREST SERVICE, U. S. DEPARTMENT OF AGRICULTURE,
LINCOLN, NEBRASKA.

STUDIES OF THE SUSCEPTIBILITY OF FORAGE GRASSES TO CEREAL SMUT FUNGI. II. A PRELIMINARY REPORT ON USTILAGO HORDEI AND U. NIGRA[1]

GEORGE W. FISCHER

(Accepted for publication March 23, 1939)

INTRODUCTION

In 1938 the writer[2] reported the natural occurrence of a smut on *Agropyron cristatum* (L.) Gaertn. and *Elymus glaucus jepsoni* Davy that had the morphologic characteristics of the organism causing the covered smut of barley, and, accordingly, the collections were assigned to *Ustilago hordei* (Pers.) K. and S. At the same time it was pointed out that on the basis of comparative morphology the 3 collections could have been equally well assigned to *U. levis* (K. and S.) Magn., cause of the covered smut of oats.

Except for a report of the natural incidence of *Ustilago hordei* on rye (*Secale cereale* L.) in Siberia,[3] the covered smut of barley has not, apparently, been reported on grasses other than cultivated species of barley. In fact, concerning the 3 collections on *Agropyron* and *Elymus* mentioned above, the question arose that, even though these collections were morphologically similar to *U. hordei* and *U. levis,* they might be physiologically and pathologically very distinct from those species. Chiefly to answer this question inoculations of barley and oats described in this paper were undertaken.

The discovery of *Ustilago hordei* on forage grasses suggested that possibly *U. nigra* Tapke,[4] which causes the black loose smut of cultivated barley, might also have some compatible hosts among the forage grasses. Preliminary inoculation experiments of several grass species with this smut are reported here.

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[1] Grass disease investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Soil Conservation Service, Section of Conservation Nurseries, and the Divisions of Plant Pathology and Agronomy of the Agricultural Experiment Station, State College of Washington.

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[3] Jackzewski, A. A. de. A new smut fungus on rye. (Translated title.) Ann. State Inst. Exp. Agronomy 3: 106–109. 1925 (Abstract in Rev. App. Myc. 5: 733. 1926).

[4] Probably the same as *U. medians* Biedenkopf used by Allison, C. C., in Studies on the genetics of smuts of barley and oats in relation to pathogenicity. Minn. Agr. Exp. Stat. Tech. Bull. 119, 1937; and Ruttle-Nebel, M. L., in Studies on barley smuts and on loose smut of wheat. New York State Agr. Exp. Stat. Tech. Bull. 221. 1934.

USTILAGO HORDEI

Inoculations of Barley and Oats

Two barley varieties, Beldi Giant (C. I. 2777) and Trebi (C. I. 936), were selected for inoculation experiments because they are generally susceptible to covered smut of barley in the Pacific Northwest. One highly susceptible variety of oats, Canadian (C. I. 1625) was used to test the possibility of the grass smuts being *Ustilago levis*. Four collections of *U. hordei* were used in the experiment: two from *Agropyron cristatum*, one from *Elymus glaucus jepsoni*, already mentioned, and one from Beldi Giant barley, grown at Walla Walla, Washington. To complete the trials one collection of *U. levis* also was used.[5] Since the amount of inoculum was somewhat scant after herbarium specimens had been taken from the collection of *U. hordei* on the grasses, all the collections were cultured on potato-dextrose agar, and combinations of pedigreed monosporidial lines of opposite sex were used for inoculum.

Four monosporidial lines (representing the 4 cells of the promycelium of the same chlamydospore[6] of each of the 4 collections of *Ustilago hordei*, and from *U. levis*) were mated on plain agar in all possible combinations, both within and between the collections. The results showed (1) the presence of only 2 sex groups in each collection; (2) that the sporidia of 1 sex group of any collection would fuse readily with the sporidia of opposite sex of all the other collections; and (3) from these fusions normal-appearing infection hyphae resulted. In determining which combinations were to be used in preparing inocula later, preference was given to those seeming to produce the most infection hyphae, although the differences in this regard were not great.

The barley and oat varieties were each inoculated with the monosporidial cultures of opposite sex within each collection and with similar cultures between the collections in all possible combinations. Zade's partial vacuum method, described by Allison,[7] was used in all the inoculations. In addition monosporidial cultures of each of the two sexes of *Ustilago hordei* were inoculated singly into the barley, and those of *U. levis* into oats. The inoculated seed was planted directly in benches of soil in the greenhouse in December, 1937.

On both Trebi and Beldi Giant barley at least some degree of infection was obtained with all inoculations involving the collections of *Ustilago hordei*, except those in which only one sex was involved. More or less infection was obtained (1) from each of the 3 grass collections of *U. hordei;* (2) with the collection from Beldi Giant barley; and (3) from crosses between monosporidial cultures of opposite sex of the 4 collections of this species in all possible combinations. No infection was obtained on oats except where inoculated with both sexes of *U. levis*. The results are presented in table 1.

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[5] Pedigreed monosporidial cultures, supplied through the courtesy of Dr. C. S. Holton.
[6] See footnote a of table 1.
[7] See footnote 4.

TABLE 1.—*Results of inoculations of Beldi Giant and Trebi barley and Canadian oats with monosporidial cultures of four collections of Ustilago hordei and one of U. levis in all possible combinations*

Inoculum	Percentage of Smutted Heads[b]		
	Beldi Giant barley	Trebi barley	Canadian oats
E-A 61 × 63[a]	34	26	0
E-A 64 × E-B 63	9	76	0
E-A 61 × E-C 51	12	17	0
E-A 63 × U.h. 62	32	37	0
E-A 64 × U.l. 911	0	0	0
E-B 61 × 63	2	44	0
E-B 61 × E-C 52	3	58	0
E-B 63 × U.h. 61	8	64	0
E-B 63 × U.l. 912	0	0	0
E-C 51 × 52	10	6	0
E-C 51 × U.h. 62	3	12	0
E-C 51 × U.l. 911	0	0	0
U.h. 61 × 62	57	26	0
U.h. 62 × U.l. 912	0	0	0
U.h. 61	0	....	......
U.h. 62	....	0	.....
U.l. 911 × 912	0	0	97
U.l. 911	....	..	0
U.l. 912	....	....	0

[a] E-A = *U. hordei* from *Elymus glaucus jepsoni;* E-B = *U. hordei* from *Agropyron cristatum* (Bozeman, Mont.); E-C = *U. hordei* from *A. cristatum* (Pullman, Wash.); U. h. = *U. hordei* from Beldi Giant barley (Walla Walla, Wash.); U. l. = *U. levis* (cultures of a virulent collection on Fulghum and Black Mesdag oats, supplied by C. S. Holton). The Arabic numerals indicate the pedigree of the monosporidial cultures. The last digit in every case indicates the origin of the culture with reference to the position, on the promycelium, of the sporidium from which the culture originated. The remaining digit or digits refer to the number given to the chlamydospore from whose promycelium the isolations were made. The numbering of the chlamydospores always begins with 5, so as to avoid confusion with the sporidium numbers, which ordinarily range from 1–4. Arbitrarily, the sporidium from the distal cell of the promycelium is designated as #1, and the numbering proceeds toward the spore.

[b] The head counts, upon which these percentages are calculated ranged from 31–82 per row. The number of smutted heads ranged from 0–35 per row.

The data presented in table 1 leave no doubt that the 3 collections of covered smut from *Agropyron cristatum* and *Elymus glaucus jepsoni* are, physiologically, *Ustilago hordei,* and that they substantiate the morphologic similarity already demonstrated. Furthermore, the same data show clearly that the collections are not related to *U. levis,* since in no instance did any combination of *U. levis* with any of the collections result in any infection on either oats or barley. The development of 97 per cent smut on Canadian oats inoculated with pure *U. levis* indicated that the inoculation method was not at fault.

Inoculations of Grasses

In order to obtain some information regarding the general susceptibility of certain grasses to *Ustilago hordei,* the 4 collections of this smut used in the above-outlined experiments were used in inoculating a series of grasses in the tribe *Hordeae.* The same technique was employed as before, using pedigreed monosporidial cultures of opposite sex, except that the inoculations were all within the 4 collections, with none between collections. Also, the inoculated seed was sown directly in the field, in 5½-foot nursery rows,[4] in the spring of 1938. The following species were inoculated: *Agropyron caninum* (L.) Beauv.; *A. cristatum, A. inerme* (Scribn. and Smith) Rydb., *A. pauciflorum* (Schwein.) Hitchc., *A. semicostatum* (Steud.) Nees, *A. subsecundum* (Link.) Hitchc.; *Elymus canadensis* L., *E. glaucus* Buckl., *E. glaucus jepsoni, E. sibiricus* L., *E. villosus* Muhl., *E. virginicus* L.; *Hordeum bulbosum* L., *H. gussoneanum* Parl., *H. jubatum* L., *H. murinum* L., *H. nodosum* L., *H. pusillum* Nutt.; *Lolium perenne* L.; *Sitanion hystrix* (Nutt.) J. G. Smith, *S. jubatum* J. G. Smith. Many of these species were represented by 2–5 selections or collections.

Probably because of late seeding, poor stands were obtained in a great many rows, and conditions for infection likewise were not good. Consequently, the results are probably quite unreliable as an index to the susceptibility of all the grasses tested. However, certain species did develop smut, and are worthy of mention: *Agropyron caninum* showed smut from collection E–B of *Ustilago hordei* (taken from *A. cristatum*); *Elymus canadensis* was smutted by collection E–C (also taken from *A. cristatum*) and by *U. h.* (collection taken from Beldi Giant barley); one collection of *Hordeum nodosum* showed smut from all four collections of *U. hordei,* and another collection of *H. nodosum* showed smut from E–A (taken from *Elymus glaucus jepsoni*), and also from E–C; *E. glaucus jepsoni* was smutted by collection E–C; *E. sibiricus* likewise showed smut from inoculation with collection E–C; *Sitanion jubatum* was smutted by collection E–C. The percentages of infection varied from about 10 per cent to 50 per cent or more, but since the stands were so poor, any tabular presentation of infection percentages would be of small value. The results do indicate, however, that certain species of forage grasses may prove to be quite susceptible to *Ustilago hordei.* This problem is being given further consideration, in which the various forage grass species will be tested for susceptibility to several collections or races of *U. hordei.*

USTILAGO NIGRA

The occurrence of *Ustilago hordei* on *Agropyron* and *Elymus* in the Pacific Northwest suggested that possibly the related species, *U. nigra,* might also find compatible hosts among the forage grasses. Accordingly, using the same technique described above for the *U. hordei* inoculations, the same series of grasses was also inoculated with pedigreed monosporidial cultures[8] of opposite sex. Only three species showed smut: *Elymus canadensis* 30 per cent, *Hordeum nodosum* 33 per cent and 50 per cent (two collections), and *Sitanion jubatum* 21 per cent. The symptoms were typical of *U. nigra* on barley, the smutted heads being dark brown and quite loose. In this respect they closely resembled the type of infection produced by certain races of *U.*

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[8] Supplied through the courtesy of Mr. Wayne Bever of the Division of Cereal Crops and Diseases, Bureau of Plant Industry, stationed at Moscow, Idaho.

bullata. Although formaldehyde-treated seed was used in the inoculations, the smutted heads (one from each plant showing smut) were microscopically examined to guarantee that the supposed appearance of *U. nigra* was not due to *U. bullata.*

SUMMARY

The natural occurrence of covered smut of barley, *Ustilago hordei,* on *Agropyron cristatum* and *Elymus glaucus jepsoni* is being investigated, and some preliminary results are presented herein.

Three collections of *Ustilago hordei* from *Agropyron* and *Elymus* and one from Beldi Giant barley were cultured on potato-dextrose agar, and pedigreed monosporidial cultures of opposite sex were used for inoculating Canadian oats and Beldi Giant and Trebi varieties of barley. As a check on the possible relationship of the collections of covered smut on the grasses to *U. levis,* pedigreed monosporidial cultures of opposite sex of this species also were used. The oats and barley were inoculated with cultures from each smut collection and also with crosses between the collections in all possible combinations. Both barley varieties were smutted, (1) by each of the 3 grass collections of *U. hordei;* (2) by the collection from Beldi Giant barley; and (3) by all the crosses between the collections. No infection was obtained on oats, except where inoculated with pure *U. levis.*

These results are thought to substantiate the morphologic similarity of the covered smut on the grasses to *U. hordei,* and to indicate a very close relationship between the two, if not to establish their identity. The results show also that the covered smut on the *Agropyron* and *Elymus* spp., probably is not related to *U. levis.*

Twenty-five species of *Agropyron, Elymus, Hordeum, Lolium,* and *Sitanion* were inoculated with each of the 3 grass collections of *U. hordei* and with a collection from Beldi Giant barley. From 10 to 50 per cent infection was obtained on *Agropyron caninum, Elymus canadensis, E. glaucus jepsoni, E. sibiricus, Hordeum nodosum,* and *Sitanion jubatum.*

The grass species inoculated with *U. hordei* were inoculated also with *U. nigra,* with 30–50 per cent smut resulting on *Elymus canadensis, Hordeum nodosum,* and *Sitanion jubatum.*

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ON THE MOLECULAR WEIGHT OF THE TOBACCO-MOSAIC VIRUS PROTEIN

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(Accepted for publication March 13, 1939)

Determination of the size of the biologically infectious unit of tobacco-mosaic virus is of sufficient fundamental importance that the various studies relative to size should be carefully scrutinized The view originally advanced that the biologically active unit was a macro-protein molecule of a molecular weight of about 17 million was reasonable on the basis of the information then available. Meanwhile, additional information has become available that should be considered in the interpretation of the data obtained by means of the ultracentrifuge. The additional information is that Fick's and Pouiselle's laws are not followed by sols of the virus protein; from inference, one may conclude that Stokes' law also is not followed.

The premise that forms the basis of the ultracentrifuge method of determining the radius of a suspended particle is that Stokes' law is valid for the material in question. According to this law, the velocity of movement of a sphere through a viscous medium depends directly on the external body forces acting on the sphere, on the reciprocal of the viscosity, on the difference in density between the sphere and medium, as well as on the square of the radius of the particle. Stokes' law was derived from the fundamental laws of hydrodynamics on the assumption that the viscous medium was continuous and infinite in extent, and that the sphere was isolated. The law is followed rigorously for a falling sphere provided the originally assumed boundary conditions are experimentally present. The law is not followed by a sphere falling through a cylinder, for example, because of the disturbances of the walls. As applied to the work with the ultracentrifuge, the sedimenting force acting on the particles is very greatly increased over the gravitational force by very rapid centrifugalization. From the known centrifugal field, the observed rate of sedimentation, the difference in density between the suspended particle and the medium, and the viscosity of the medium, the *effective* radius of the particle may be calculated. Reliable results for the radius may be obtained if the individual particles sediment as if each were alone in a viscous medium of continuous and infinite extent. There is no reason to doubt that these conditions obtain for some protein preparations. Whether or not the particles in question are molecules is an entirely different matter.

No one has derived an equation for the sedimentatien of a nonspherical particle through a viscous medium, so great are the mathematical difficulties involved. It is for this reason that the "asymmetry constant" is used in ultracentrifugal work for correcting the weights of particles believed to be non-spherical. On the arbitrary assumption of an asymmetry constant of 1.3 (1) for the virus protein, a particle weight of about 17 million was obtained from centrifuge data. Later, viscosity data were interpreted as indicating that the protein particles were 35–37 times as long as they were thick (2) (5); from these relative dimensions, an asymmetry constant of 2.52 was calculated (5). The value of 17 million was then corrected to 43 million.

The agreement between the size of the protein particle, as determined by sedimentation studies on the one hand and diffusion studies on the other, is very poor. A particle weight of about 1,300 million is obtained from the diffusion constant of 3×10^{-8} (6). In this connection recourse again was made to a correction on the assumption of a rod-shape particle having relative dimensions of 1:37, and the weight was corrected to 90 million. On combining sedimentation and diffusion data, a value of 59 million may be calculated. On the surface, the agreement between 43, 59, and 90 million may seem to be not so very bad, but it is fortuitous. If, on the other hand, one considers the diffusion constant of 4.5×10^{-9} (3), the discrepancy is large, indeed.

The mathematical expression for the phenomenon of diffusion is known as Fick's law. It was derived from the fundamental laws of hydrodynamics, on the assumption that the diffusate might be considered as a perfect but compressible fluid. Rather good confirmation of the law has been obtained with colloidal solutions, and, in general, colloidal solutions with normal osmotic behavior obey the normal laws of diffusion. The law is not followed by sols made up of the virus protein (3, 6). The dynamics of the diffusing particles is abnormal, indicating that abnormally large forces between the particles are at play. The suggestion has been advanced that the abnormality in the diffusion process is attributable to an entanglement of the very long protein particles, inasmuch as calculations may be carried out to show that particle lengths are of the same order of magnitude as the interparticle distances (6, 3). This contention is not supported by viscosity studies (4). It will be shown on another occasion (4) that the abnormalities are not because of shape alone.

The Brownian movement of the protein particles is not normal, if one may argue from analogy. It is well known, for example, that quiescent thixotropic bentonite clay sols, as viewed through an ultramicroscope, do not present a picture of incessant motion, but rather the field resembles the sky on a clear night, with the exception that there is an occasional flash of light as an individual particle rotates. In this connection it may again be pointed out that the assumption in applying Stókes' law in the ultracentrifugal studies is that each particle is acted on only by normal external body forces, and by the forces of friction attributable to the viscous nature of the solvent.

A further consideration is that the viscosity of the virus protein in water or phosphate buffer is anomalous to a very marked degree and is typically that shown by thixotropic systems. In other words, Pouiselle's law is not followed. The very great increase in apparent viscosity as the shearing stresses along the tube of the capillary are decreased (3) is suggestive of a spontaneous repair of broken structure. The anomalous viscosity of soaps or starch, usually cited as examples of materials showing anomalous viscosity, does not compare with the marked anomaly shown by the virus protein. The anomaly shown by a .05 per cent sol of the virus protein in phosphate buffer at pH 7 is greater than that shown by a 1 per cent sol of sodium oleate.[1] Because of the extreme anomaly, it is not possible to determine the ratio of length to thickness of the virus particles by means of viscosity studies either in water or dilute solutions of electrolytes Consequently, there is no justification for the correction of either diffusion or sedimentation data on the basis of a ratio of thickness to length of the particle of 1: 35–1: 37.

The "monodispersity" of the protein sols as observed in the ultracentrifuge does not present any difficulties in the present discussion. A test for monodispersity is the linearity between log c and x^2, where c is the concentration at the point x and x is the distance from the center of the centrifuge. Such a linear relationship would be characteristic of a gel or any elastic system that obeyed Hook's law.

Fick's law is fundamentally sound, and the development of the kinetic theory for a centrifugal field has, of course, been confirmed. It is legitimate, therefore, to consider that the discrepancy in the values for the "molecular" weights of the virus protein in water or phosphate buffer as obtained by means of diffusion and sedimentation as significant. The interpretation of the observed discrepancy is somewhat obscure, but in view of the viscosity behavior, it is very probable that the lack of agreement may be interpreted in terms of dynamically interdependent protein aggregates. One is rather inclined to suspect that the premise forming the basis for the use of Stokes' law for determining the radius of the virus protein molecule is false. It may be said that the molecular weight of the protein is not surely known.

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UNUSUAL FEATURES IN THE BEHAVIOR OF SCLEROTIA OF PHYMATOTRICHUM OMNIVORUM

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(Accepted for publication March 2, 1939)

As a result of extensive investigations by various investigators on the morphology and life history of the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Dug., 3 stages in its development are now known: (a) the Ozonium or vegetative stage, which spreads through the soil and attacks the roots of susceptible plants; (b) the sclerotial or resting stage, which aids materially in enabling the fungus to survive unfavorable conditions; and (c) the conidial or "spore mat" stage, which frequently occurs above ground in badly infested areas under suitable climatic conditions and the function of which is still unknown.

The discovery by King and Loomis[2] of the sclerotial stage of *Phymatotrichum* in laboratory cultures and the subsequent findings of viable sclerotia in the soil of infested areas in Texas, Arizona, and California,[3, 4, 5] were important additions to our knowledge of the fungus and have stimulated much of the subsequent investigation. The sclerotia develop as elongate swellings of the mycelial strands and attain their maximum growth within 10 to 14 days, the mature sclerotia being round to ovoid, ellipsoid, variously constricted, and having the general appearance of beads on a string. King and Loomis[2] state that the sclerotia are formed by the division and growth of cells of the strand and that this division takes place in the large central hyphae as well as in the smaller cells that surround them in the strand.

In maturing, the sclerotium changes from white to creamy buff and finally to a dark reddish brown or black. The outermost filaments, "acicular hyphae," shrivel and form a fuzzy covering beneath which develops a dark-color rind 2 or 3 cells in thickness. In this rind the cells are small, roundish, heavy-walled, and crowded close together. In surface view there is a labyrinthine arrangement of the cells, and from these the acicular hyphae arise The central mass appears as a homogeneous pseudoparenchyma made up of intertwined, short-celled hyphae that are much thicker than ordinary hyphae. These thick hyphae are formed on the primary

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[1] The writer takes pleasure in acknowledging the helpful suggestions and criticisms by Dr. H. A. Rodenhiser, of the U. S. Department of Agriculture.

[2] King, C. J., and H. F. Loomis. Further studies of cotton root rot in Arizona, with a description of a sclerotium stage of the fungus. Jour. Agr. Res. [U.S.] 39: 641–676. 1929.

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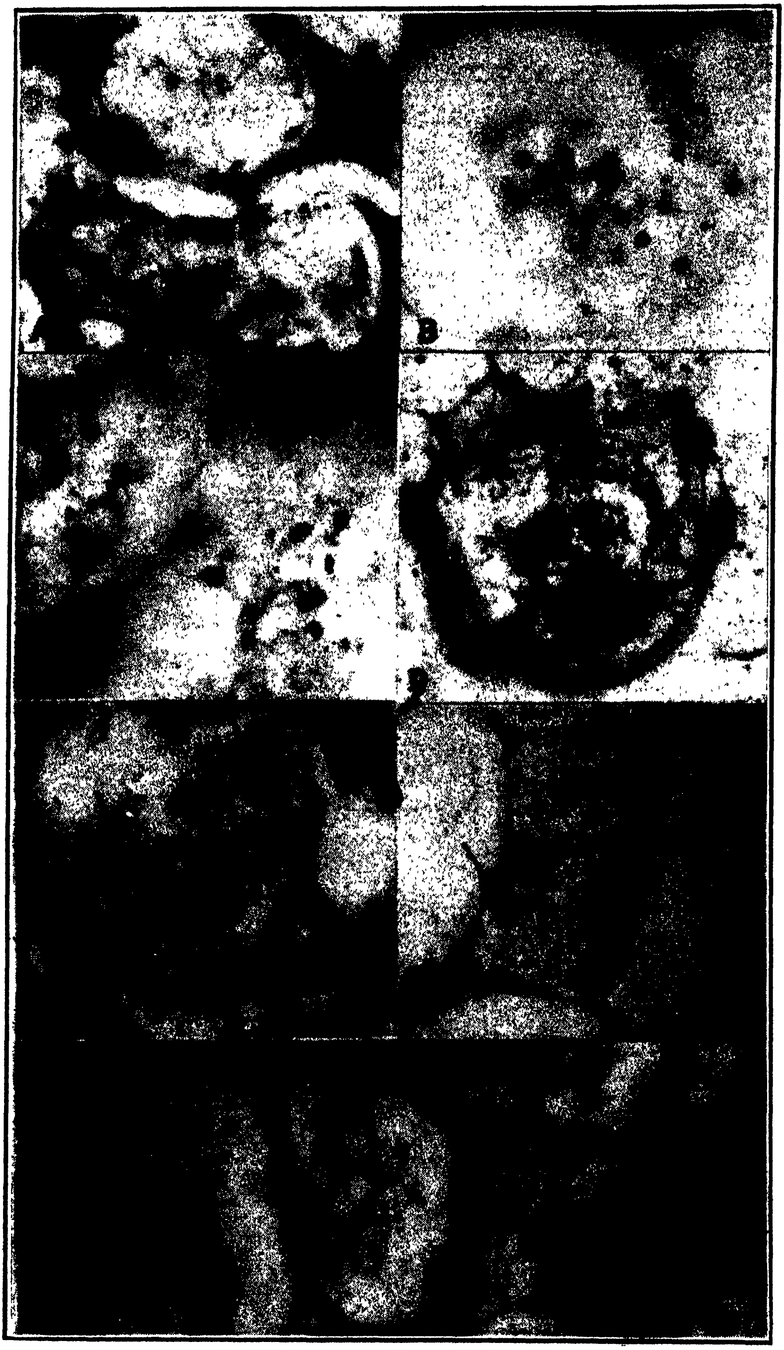

FIG. 1. Sclerotial cells of *Phymatotrichum omnivorum*, showing various stages of disorganization and the beginning of reorganization. A. Resting cells. B. Enlarged nuclei. C. Disorganizing nuclei. D. Disorganization complete. E, F. Early stages of reorganization. G. Three stages of disorganization in adjacent cells. A, D, G, approx. ×560; B, C, approx. ×1120.

mycelium; they are storage organs for reserve food material, and so far as is known, they remain dormant for various periods.

The sclerotia produced by many fungi, when placed under favorable conditions, ultimately expend their reserve food in the development of reproductive structures from primordia located just beneath the rind, which develop at the expense of the remainder of the sclerotium. In contrast to this behavior every cell in the sclerotium of *Phymatotrichum* seems capable of reproduction. However, they have never been known to germinate and give rise to reproductive structures; instead, a sterile mycelium is produced by "vegetative sprouting." This process continues as long as any part of the sclerotium remains viable. As mentioned previously, the sclerotia are made up of specialized hyphal cells, and the vegetative sprouting has been considered as a continuation or resumption of growth of these cells. However, a detailed study at the time of sprouting shows a very unusual behavior. The new hyphae do not arise as branches or elongations of the resting cells, but appear to be formed actually inside the cells, whose contents apparently go through a process of disorganization and reorganization that results in the formation of new hyphae instead of sporophores. Each of these newly formed hyphae can produce a mycelium of the fungus.

If a mature sclerotium that has just been recovered from a root-rot-infested field be sectioned, stained, and examined microscopically, the structure will appear homogeneous, with no trace of primordia. Each cell of the pseudoparenchyma is multinucleate and appears to be dormant (Fig. 1, A).

The sequence of events within the cells, based upon the material observed so far, is as follows: The nuclei become somewhat enlarged, as though preparing for meiotic division; then disorganization begins. When disorganization is complete, the whole cell has a different staining quality and a quite different appearance from what it originally had. Small, dark-staining bodies, possibly nucleoli, are visible throughout the cell at this stage. There seems to be no definite order in which individual cells go through this activity; hence, the time required for the completion of the process will be difficult to determine. In figures 1 and 2 are shown the different phases of the process, *i.e.*, the swollen nuclei, the disorganizing nuclei, the completely disorganized nuclei, and the various stages of reorganization. After complete disorganization of the nuclei, two of the dark-staining bodies may come together, as shown at point of arrow (Fig. 1, E), a localized area of cytoplasm becomes increasingly more dense (Fig. 1, F), and hyphae are then formed (Fig. 2, J, K, L). It is suggested that the activity of reorganization may be caused by the coming together of the bodies as shown in figure 1, E. When the hypha is completely formed, the "mother" cell wall breaks and the new hypha emerges. On emergence from the sclerotial shell some of the hyphal tips have peculiar branches or appendages that appear to become detached after the hyphae have emerged (Fig. 2, M, N). The newly-formed hyphae are multinucleate and resemble in every respect those of the vigorously growing vegetative mycelium of the fungus. They give the sclerotium

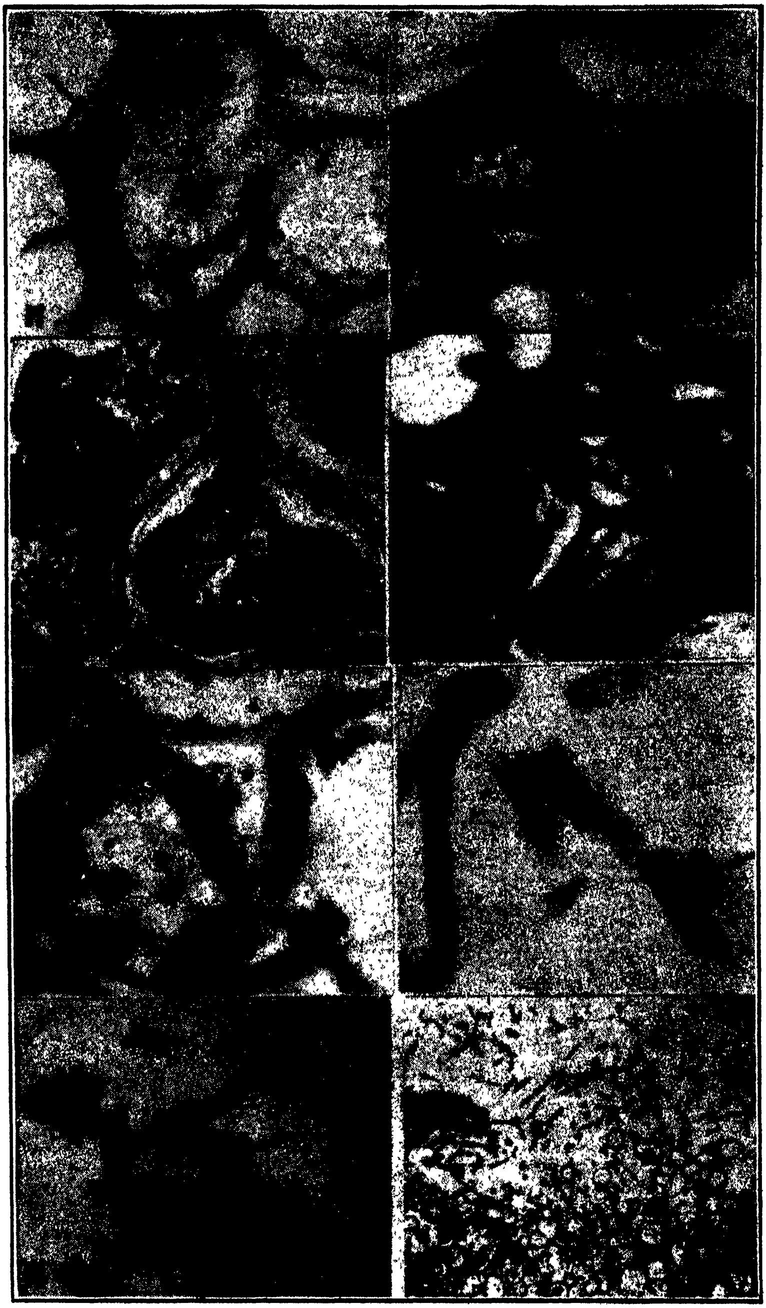

FIG. 2. Sclerotial cells of *Phymatotrichum omnivorum* showing reorganization completed and appendages on some of the hyphae thus formed. H–K. Formation of hyphae. L. Mature hyphae within the walls of "mother" cell. M. Appendage or sprout on tip of emerging hyphae. N. Appendage apparently becoming detached. O. Section of sclerotium showing emerged hyphae and abundant round bodies similar to the appendages in M and N. H–N, approx. × 560; O, approx. × 280.

the bristly appearance typical of "germinating sclerotia" of *Phymatotrichum.*

The meaning of the processes here described is not clear, but the processes themselves have been studied thoroughly. The material has been examined and the writer's interpretations confirmed by several competent observers. Final conclusions regarding the significance of the observations must await further cytological investigations, which are now in progress.

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FRENCHING OF TOBACCO

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(Accepted for publication February 15, 1939)

Although frenching of tobacco was first described by John Clayton in 1688 (3) and has been recognized as a physiological disease since 1914 (2), the cause of the disease is still unknown. One hypothesis that has been advanced is that frenching may result from the toxic action of thallium. Several workers (4, 6, 1) have shown that the symptoms of thallium toxicity induced under controlled conditions simulate very closely those of natural frenching. Final proof that the disease is caused by thallium is lacking because it has been impossible to detect thallium in frenched plants or in field soil on which the disease occurs. Recent studies using the spectrograph are of interest in this connection.

Spectrographic analysis has been employed as a method for the detection of thallium in Turkish tobacco plants (*Nicotiana tabacum* L.). Approximately 25 mg. of dried plant material were placed on purified carbon electrodes and then arced with a direct current of 110 volts. The resultant spectra were photographed with a Hilger quartz spectrograph using 10-inch plates. The spectral line with a wave length of 2767.9 Ångstroms was found to be most useful for the detection of thallium. The results obtained with plants grown under greenhouse conditions are presented in table 1. These

TABLE 1.—*Spectrographic analysis of thallium in tobacco plants receiving various amounts of thallium*

Plants grown in	Thallium added (p.p.m.)	Disease symptoms	Spectrographic test
Sand	0	None	–
"	.025	None	–
"	.05	Only lower leaves slightly yellow	–
"	.1	Faint mottling throughout plant	+
"	.2	Slight strap-leaf formation	+
Field soil	0	Chlorosis typical of frenching	–
Composted soil	0	None	–

tests were first carried out in September, 1938, and later repeated in December, 1938, with similar results. Plants grown in nutrient sand cultures and supplied daily with a balanced, 3-salt nutrient solution at a concentration of 0.2 atmospheres gave a negative test for thallium. However, plants receiving the same nutrient solution together with sufficient thallium to produce only faint symptoms of toxicity (0.1 p.p.m. of thallium as $TlNO_3$) were able to absorb detectable amounts of thallium from the solution. No thallium could be detected either in the tops of frenched plants grown in field soil or in healthy plants grown in composted soil.

These data suggest that frenching and thallium toxicity are two distinct physiological diseases. However, if the two diseased conditions are caused by different agents, it is surprising that they are so similar not only with respect to the symptoms produced on Turkish tobacco, but also with regard to the methods by which they are controlled (5, 6). Furthermore, a water extract of a non-toxic soil, collected near a natural frenching area, does not produce frenching until supplemented by the additive effect of a non-toxic amount of thallium (6). Root symptoms of both diseases are also similar (5, 6).

It is not improbable that the toxic action of thallium may be exerted on the root, thereby altering its metabolism, and giving rise to some disturbance that affects the top. Since it is not known in what form thallium may occur in the soil, it is conceivable that it may be present as some complex compound that is not so readily translocated into the top as the thallium salts used in experimental studies. If this were true, the spectrographic detection of thallium only in those plants treated with $TlNO_3$ would not necessarily eliminate the possibility that thallium may be the cause of frenching.

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GROWTH AND VARIABILITY OF STEREUM GAUSAPATUM IN CULTURE[1]

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(Accepted for publication February 28, 1939)

INTRODUCTION

Stereum gausapatum Fries has recently been found important as a cause of heart rot of oak (2, 3, 6). This discovery depended upon cultural identification of the fungi isolated from diseased trees (2, 4). The known importance of this fungus, together with the fact that it exhibits peculiar behavior in culture, led the writer to investigate its biology. The present paper, however, is concerned primarily with growth and variability of this fungus in culture. Bergenthal (1) describes the growth of *S. gausapatum,* but makes no suggestion of any irregularity or variation such as has been noticed by Davidson[2] and studied by the writer.

SOURCE OF CULTURES

A total of 58 isolates was employed in this study. Cultures of 19 isolates were obtained from the Division of Forest Pathology, U. S. Department of Agriculture, Washington, D. C.[3] The writer established 3 isolates from local collections. In addition to the above cultures, none of which were started from single spores, 36 monosporous cultures were established from spores of a single fruiting body. The various cultures will be referred to by the numbers presented in table 1.

GROWTH AND VARIABILITY IN CULTURE

Transfers from old agar cultures or from cultures derived from old agar cultures through a few successive transfers may grow but a few millimeters and then cease growing, or they may grow in a lobed or sector fashion, or indefinitely at a uniform rate with an even margin. The latter type of growth will be designated as normal. The behavior varies widely with the different isolates. Before carrying on any other cultural studies of this organism, it was necessary to discover how to produce cultures of normal growth.

It was found that successive transfers, made from the most vigorously growing portion of the mycelium, tended to grow better than the immediate parent mycelium. Transfers from an old culture of an isolate that is nor-

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[1] A revision of a section of a thesis presented to the Graduate School of the Ohio State University in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The writer wishes to acknowledge his indebtedness to Dr. W. G. Stover and other members of the faculty of the Ohio State University for various types of assistance, and to Mr. R. W. Davidson, Division of Forest Pathology, U. S. Department of Agriculture, for cultures and helpful suggestions.

[2] R. W. Davidson, personal communication (Nov., 1936) states "that growth of this fungus in culture is often irregular and uncertain."

[3] The cultures were provided through the kindness of Ross W. Davidson.

TABLE 1.—*Source of isolates of Stereum gausapatum*

Writer's number	Host	Date of collection	Locality	Forest Pathology number
1[a]	Oak	Oct., 1936	Jackson, Ohio	
2[b]	Oak	Dec., 1933	Edinburg, Va.	77A
3	Oak	Jan., 1933	State College, Pa.	56496–S
4[c]	Oak	Nov., 1936	Columbus, Ohio	
5	White oak	Apr., 1933	Portland, Conn.	58520–R
6	Oak	Oct., 1933	Sunset Hills Va.	58546–R
7	Oak	Oct., 1933	Sunset Hills, Va.	58546–S
8	Oak	Oct., 1933	Sunset Hills, Va.	58547
9	White oak	Nov., 1933	Hyde Park, N. Y.	58554–R
10	White oak	Nov., 1933	Hyde Park, N. Y.	58554–S
11	Oak	Nov., 1933	Hyde Park, N. Y.	58555–R
12	Oak	Nov., 1933	Hyde Park, N. Y.	58555–S
13	White oak	Nov., 1933	Hyde Park, N. Y.	58556–R
14	White oak	Nov., 1933	Hyde Park, N. Y.	58556–S
15	Oak	Jan., 1934	Columbia Furnace, Va.	58577–S
16	Oak	Dec., 1934	State College, Pa.	59133–R
17	Oak	Dec., 1934	State College, Pa.	59138
18	Black oak	Feb., 1935	Shenandoah, Va.	59152–R
19	White oak	Sept., 1935	Cobalt, Conn.	67956–R
20	White oak	Sept., 1935	Cobalt, Conn.	67956–S
21	Oak (?)	Oct., 1936	Cadillac (?), Mich.	71433–S
22[d]	White oak	Oct., 1937	Columbus, Ohio	
S–1 to S–36[e]	Oak	Nov., 1936	Columbus, Ohio	

[a] This isolate was established from sporophore tissue collected by the writer.

[b] This isolate was obtained from decaying wood in a living tree. The S after the Forest Pathology numbers indicates that the culture was obtained from basidiospores and the R indicates that the culture was started from the mycelium in decayed wood. Since this paper was accepted for publication, R. W. Davidson and L. O. Overholts have decided that this isolate is *S. rameale* and not *S. gausapatum*, as originally identified. The sporophores from which most of the Forest Pathology cultures were obtained were identified by L. O. Overholts.

[c] This isolate was established from sporophore tissue collected by the writer.

[d] This isolate was established from basidiospores collected by the writer.

[e] The 36 monosporous isolates are designated as S–1 to S–36, respectively.

mally a rapid grower, *e.g.*, isolates 3 and 12, grow much better than transfers from normally slow-growing isolates, such as 2, 5, 9, etc. (Fig. 1). A transfer from an old culture of isolate 12 will usually grow uniformly, but more slowly than normal. After 2 or 3 successive transfers of isolate 12, made at short intervals (3–5 days), a normal culture may be obtained. In extreme contrast, a transfer from an old culture of isolate 2 often will grow only a few millimeters, or not at all. In order to obtain a vigorous culture of this isolate it was found necessary to make a series of successive transfers at intervals of 3 to 5 days over a period of several weeks. The number of transfers necessary depends somewhat upon the past history of the culture. The difficulty involved in producing normal cultures of the various isolates ranges between the two extremes.

The only report in the literature pertaining to the appearance of *Stereum gausapatum* in culture is Bergenthal's (1) key to the species of *Stereum*, which is based on mycelium as grown on "Brot, Bromalzagar, und Holz." He describes it as "Weiss, kompakt, später braune Zonen bildend." The present study leaves no doubt as to the inadequacy of the above description.

A comparative study of mycelia of the various isolates was made by growing the fungus in 90-mm Petri dishes containing approximately 25 cc. of potato dextrose agar.[4] From the 58 isolates in culture, 12 were selected to represent the variation present. Vigorous cultures were produced by the method described above. Ten daughter cultures were prepared from each of the 12 vigorously growing mycelia. The plates were uniformly inoculated at one edge with mycelium taken from the margin of the culture. These plates were incubated in darkness at 25° C. (The plates were not inverted.) On the 7th day they were examined. Very little difference could be seen between the 10 plates of any one isolate. One plate of each isolate was photo-

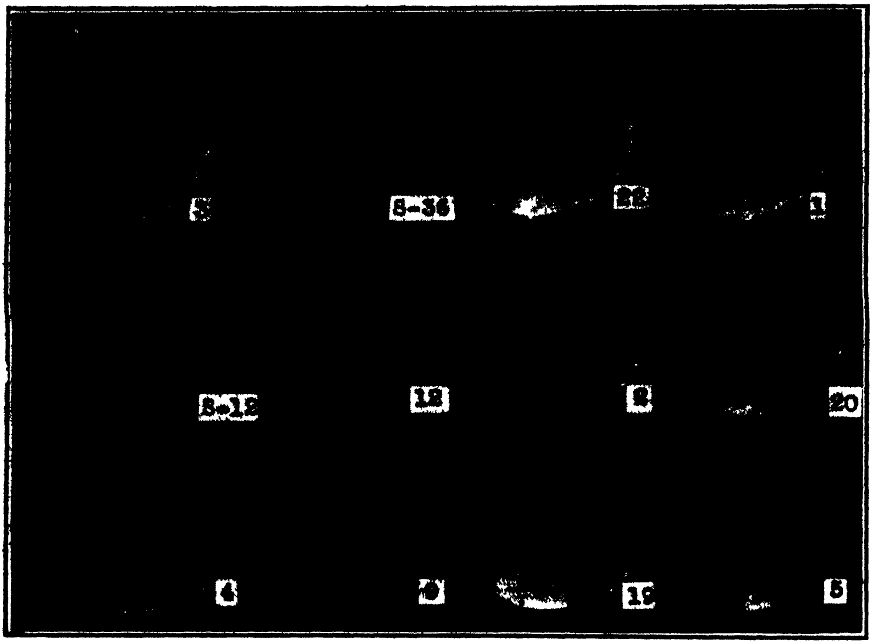

FIG. 1. Seven-day-old cultures of 12 isolates of *Stereum gausapatum* showing differences in character and rate of growth. (See table 2 for description of cultures.)

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[4] The potato dextrose agar was prepared as follows: (1) wash and slice 200 g. of potatoes into 500 cc. of tap water, (2) add 18 g. of agar agar to 500 cc. of tap water, (3) autoclave the above for 30 minutes at 15 lb. pressure, (4) add broth from potatoes to agar solution, also add 20 g. of dextrose, (5) autoclave 20 min. at 15 lb. pressure, (6) put into flasks and sterilize. The final pH was found to be 5.2.

graphed (Fig. 1), studied, and discarded. The others were returned to the incubator for further development. The striking, but characteristic, differences in growth rate noticeable at this time are clearly shown in figure 1. The procedure was repeated on the 16th and again on the 44th day.

In order clearly to describe the mycelia of the various isolates and show the similarities and differences, figures 1, 2, and 3 are presented, together with a brief description[5] (Table 2) of each culture photographed.

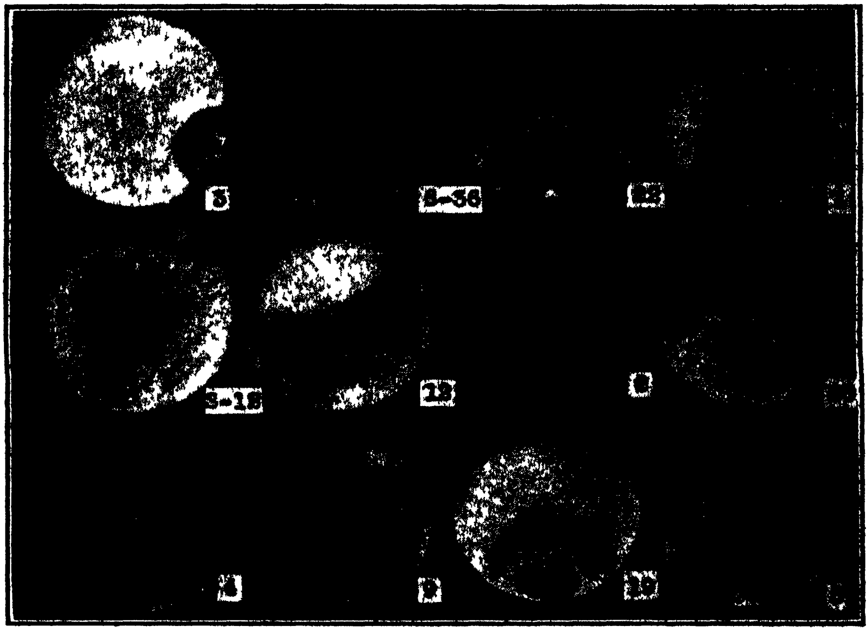

FIG. 2. Sixteen-day old cultures of 12 isolates of *Stereum gausapatum*. (See table 2 for description of cultures.)

DISCUSSION

Cultures of the various isolates have been under observation by the writer for nearly 2 years. During that time there has been no indication that the variations shown are not permanent characteristics of the respective mycelia.

The most striking variation was shown by cultures of isolate 3 in the production of the "depressed area" (Fig. 2). The only other suggestion of such a feature was shown by the mycelium of one of the 36 monosporous cultures. The trait was not pronounced but did show a definite similarity. Cultures of Isolate 12 were distinctive because the mycelium never developed into a tough mat nor did it ever become dark colored (Fig. 3). Cultures of this isolate have been kept at room temperature for periods of approximately 6 months, but the mycelium remained white and cottony. Cultures of Isolate S–36 stood out by virtue of their rich "orange" color, concentric zona-

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[5] In the descriptions all color nomenclature is that of Ridgway (5) unless enclosed by quotation marks.

TABLE 2.—*Descriptions of typical cultures of twelve representative isolates growing on potato dextrose agar*

Isolate	7-day-old culture (Fig. 1)	16-day-old culture (Fig. 2)	44-day-old culture (Fig. 3)
3	White, except for Naples yellow depressed area surrounding point of inoculation with spots of mustard-yellow and white. Depressed area a thin tough mat; fluffy aerial mycelium elsewhere; margin thin.	Mat of depressed zone thicker, raw-sienna color; elsewhere a white to cream and cottony with no compact surface layer. (**Note:** Through accident all but one culture of this isolate was lost at this time; hence it served for this and the succeeding figure.)	Similar to 16th day, but with a second narrow depressed zone surrounding the first. Around much of the perimeter the mycelium had grown onto the lid; hence the raggedness seen in the photograph.
S–36	Light-ochraceous-buff; pile deep, appearing as though combed radially; no dense surface mat; margin conspicuously of same thickness as older region and very even.	Orange-buff; depth of pile varies in concentric zones; small areas of dense surface mat near inoculation point.	Warm-buff; aerial mycelium more dense; patches of dense orange-buff surface mat, bearing drops of clear fluid near point of inoculation.
22	Colors zonate, from center out: light-buff, pale-yellow-orange, nearly white, light-buff and white; pile medium length and denseness; older portion with chamois-like mat; margin very uniform and more dense than most.	Cinnamon-brown to light-buff with patches of chestnut drops occurring on the cinnamon-brown area; the older region consists of a fragile, compact surface mat.	Colors darker than before; mat thicker and more extensive; surface dotted with wart-like fungous growths; much "coffee"-color secretion on the mat.
1	Pale orange-yellow in older portion, light-buff to white at border; pile short; surface mat very thin and fragile.	Central 1–2 cm. light-ochraceous-salmon, thick chamois-like mat; then a wider zone of low, compact, light-ochraceous-buff mat; finally, a wide zone of deep, dense, cottony light-buff mycelium.	"Brownish" central zone, then light-buff, followed by 4–5 cm. of ochraceous-orange to lighter, and finally a band of light-buff; pile very short; entire area covered by a dense chamois-like mat.
S–12	White; aerial mycelium fluffy and deep; no compacting; margin thin.	Capucine-buff along sides, lighter shades of buff in center of culture to white at edge opposite point of inoculation; aerial mycelium deep and cottony, especially along sides; no compact mat	Pale yellow-orange and light shades and tints of buff, with a patch of "brown" to almost black along each side. No compact mat has ever been formed by this isolate.
12	White; deep, fluffy aerial mycelium; no tough surface mat. A pillow-like growth covers the inoculum; margin thin.	White to light-buff; deep cottony aerial mycelium, extending slightly onto lid.	Same as on 16th day, except for increased growth onto lid of dish. No tough mat or dark color has been found in any agar culture of this isolate.

TABLE 2.—(*Continued*)

Isolate	7-day-old culture (Fig. 1)	16-day-old culture (Fig. 2)	44-day-old culture (Fig. 3)
2	In contrast to all others studied, the mycelium is largely submerged. Only a sparse pile shows above the agar. The older portion is light-buff due to the slight aerial growth.	Aerial portion (central half), light-buff to cinnamon-brown; surface mat very thin and fragile, bearing short pile. Note submerged mycelium.	Entire area now covered by a delicate surface mat, bearing short sparse, pile. A mixture of grays and browns with yellow-orange. Raised growths in older portion, bearing drops of "amber" fluid.
20	Light-buff to white; aerial mycelium fluffy with "lumps"; no compact layer next to agar.	A thick, tough, pale yellow-orange mat extends to middle of dish, then a narrow zone of capucine-buff. The remaining portion of the mycelium is white and fluffy, with "lumps."	Pale orange-yellow throughout; thick, tough mat covers entire agar surface. Mat thicker than on 16th day.
4	Light-buff to slightly darker in oldest portion; pile short and uniform, appearing as though combed; oldest part of mycelium compacted into a chamois-like mat; margin thin.	Light-buff to ochraceous-buff; pile as on 7th day; mat ranges in thickness from nearly bare agar to a 2 mm.-thick chamois-like mat in youngest region.	Light-buff to pinkish-buff with spots of cinnamon-buff; similar to 16th day, but with much thicker mat in oldest and youngest regions; agar remains nearly bare in central region.
9	Light-buff near point of inoculation, white elsewhere; pile short and even; a fragile surface mat in oldest portion; margin very thin.	Cinnamon-buff around point of inoculation, then a zone of sayal-brown bearing many drops of "chestnut" fluid; color gradually lightens to pinkish buff in youngest region. A thin, tough mat covers entire area. One centimeter of agar remains bare.	No significant change in appearance; mat thicker; more secretion; colors darker and another zone of "brown" bearing drops of fluid has formed. Four or five mm. of agar remain bare opposite point of inoculation.
19	Chalky white to white; pile short; older half of mycelium a very thin chamois-like mat with a short pile; margin moderate in thickness.	From center outward; "grayish brown," light-buff, cinnamon and light-buff; growth short and dense; chamois like mat extends almost to margin of mycelium.	Oldest portion buckthorn-brown, then cinnamon-brown bearing drops of fluid, then a zone of various browns and buffs; youngest region light-buff. Similar to 16th day but chamois-like mat thicker.
5	Light-buff around point of inoculation, then ochraceous-tawny, followed by light-buff; pile, medium depth, with small "lumps" near margin; very thin chamois-like mat extends to 1 cm. from margin.	White around point of inoculation, then raw-umber, Saccardo's umber, raw-umber, Saccardo's umber then white in the order named. The central region consists of a thick chamois-like mat. Note white "lumps" in younger portion.	Central area larger, darker color, mat thicker, radial wrinkles present; on either side of the point of inoculation there are ochraceous buff wart like bodies bearing drops of "amber" fluid. Light area cream color, thick and tough.

tion (Fig. 2), and the dense, abrupt margin of the advancing mycelium (Fig. 1). Isolate 2 stood alone in the extreme difficulty involved in producing a "normal" culture from an old one. It was the only isolate studied whose mycelium normally advanced as a submerged mycelium (Fig. 1). Old cultures of Isolate 5 were quite recognizable because of the dark, compact mycelial mat (Fig. 3). Cultures of the other 7 isolates were less distinctive, but they had sufficient individuality to be distinguished on the basis of their appearance.

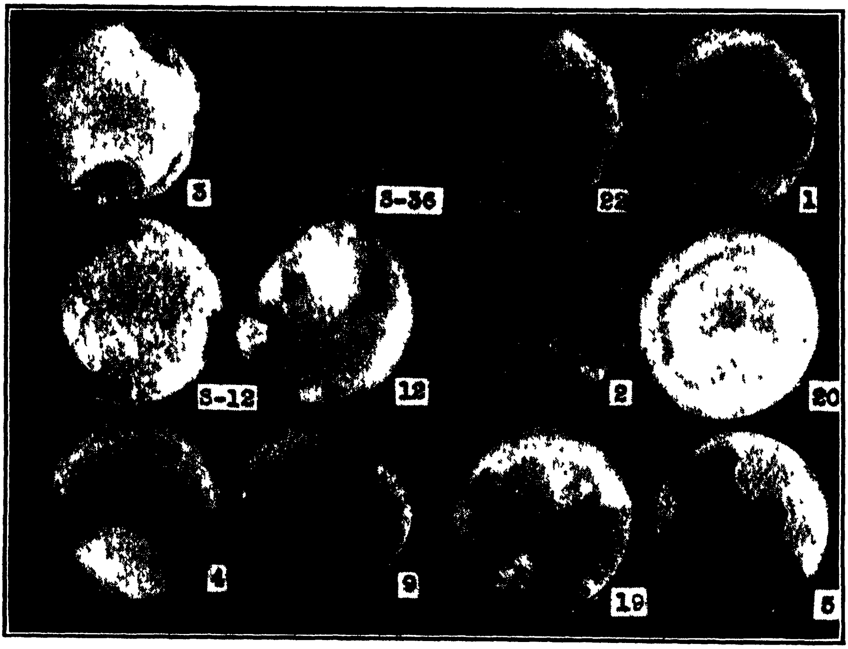

FIG. 3. Forty four-day-old cultures of the same 12 isolates of *Stereum gausapatum* as shown in figures 1 and 2. (See table 2 for description of cultures.)

The 36 monosporous cultures, all of which were from spores of a single fruiting body, showed much less variation than the "spore-mass" cultures or the cultures from decay.

It should be mentioned also, that at various times, cultures of all these isolates have been grown on Difco malt agar alongside cultures of the same isolates on potato-dextrose agar, and that no essential difference was to be noted between the mycelia growing on the 2 substrata.

The demonstration of definite strains of *Stereum gausapatum* opens a large field for further research How many distinct strains are there? How are the various strains distributed geographically? Are they all equal in their wood-destroying powers? Are they correlated with variation in sporophore characteristics? Do the various strains differ in physiological behavior? These and other questions should be worthy of investigation.

SUMMARY

This investigation deals with the growth and variability in culture of *Stereum gausapatum,* cause of heart rot of oak. The cultures used represent collections made at various points throughout the Northeastern United States.

Transfers from old agar cultures or from cultures derived from old agar cultures grew in an unpredictable fashion. It was found that a sufficient number of successive transfers, made at short intervals, gave rise to cultures of predictable behavior. The number of successive transfers necessary to produce such results varied widely with the 58 isolates studied.

An intensive study of 12 selected isolates was made by growing the fungus on potato-dextrose agar at 25° C. The cultures varied widely in their growth rate, as well as in the character and color of the mycelium.

One of the most striking cultural variations was shown by the mycelium of one isolate in the production of a depressed zone surrounding the point of inoculation. Cultures of some isolates were distinctive because they never became dark colored nor compacted into a tough mat, but always remained cottony and nearly white. The mycelia of others stood out by virtue of their rich color, concentric zonation, type of margin of the advancing mycelium, or other characteristics. Only one isolate regularly advanced as a submerged mycelium. Others were characterized by dark colored mycelial mats. The various isolates remained constant in their cultural characteristics during nearly 2 years' duration of the study.

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THE BEHAVIOR OF POJ 2878 SUGAR CANE IN RELATION TO FIJI DISEASE AND TRANSMISSION OF THE VIRUS BY NYMPHS OF PERKINSIELLA VASTATRIX[1]

GERARDO OFFIMARIA OCFEMIA AND MARTIN S. CELINO

(Accepted for publication November 27, 1938)

On February 25, 1936, stalks of apparently healthy POJ 2878 sugar cane were carefully selected from the field of the Department of Agronomy of the College of Agriculture at Los Baños for use in Fiji-disease-transmission experiments. Each of the stalks contained 11 nodes. The nodes of each stalk were examined carefully in the laboratory in order to be sure that they were free from fungus and insect injuries. The nodes were then numbered consecutively from the base upwards, the internodes were girdled, and the canes were treated and planted in the manner described by Ocfemia[2] in 1934. In order to protect the young cane shoots from infection the potted one-node cuttings were placed in insect-proof chambers where their shoots were allowed to emerge and develop.

Observations on the shoots that developed from each of the eyes were made on March 23, 1936, and on September 25, 1937. Although the stalks of POJ 2878 collected from the field were apparently healthy, the shoots of 2 of the stalks showed a behavior in relation to Fiji disease similar to that reported in 1929 by Stahl and Faris[3] for sugar-cane mosaic in Cuba. These 2 stalks were apparently infected with the disease during the growing season, but the virus had not been able to spread throughout the stalks. For convenience in handling, these 2 stalks were designated A and B. Eyes 2, 3, 4, and 5 of stalk A, and buds 5, 8, and 9 of stalk B produced shoots showing symptoms of Fiji disease. Nodes 1, 6, 7, 8, 9, and 10 of stalk A, and nodes 1, 2, 6, 7, and 11 of stalk B, produced healthy shoots. The stalks of hills originating from each of the normal shoots were still free from Fiji disease on July 26, 1938. The bud of node 11 of stalk A and the eyes of nodes 3, 4, and 10 of stalk B did not germinate.

As the freedom from symptoms of Fiji disease might be due either to tolerance for the virus or to inability of the infective material to multiply and spread throughout the stalks, experiments were conducted to determine (*a*) whether or not the apparently Fiji-disease-free canes from stalk A and stalk B of POJ 2878 do not contain the infective principle, and (*b*) whether or not they can be infected with the disease-inducing virus. Along with these experiments, tests were made to find out what stages of the life history of *Perkinsiella vastatrix* Breddin (Delphacidae) can transmit the causative virus, whether or not the young that hatch from eggs laid by viruliferous mothers carry the virus, and the shortest time of feeding on experimental canes necessary to effect transmission.

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[1] Contribution from the Experiment Station of the College of Agriculture at Los Baños, Laguma, Philippines. Published with the approval of the Director.

[2] Ocfemia, G. O. An insect vector of the Fiji disease of sugar cane. Amer. Jour. Bot. 21: 113–120. 1934.

[3] Stahl, C. F., and James A. Faris. The behavior of the new POJ canes in relation to sugar-cane mosaic in Cuba. Trop. Plant Res. Found. Bull 9: 3–12. 1929.

MATERIALS AND METHODS

In these experiments the materials used included healthy and Fiji-diseased POJ 2878 sugar cane, healthy canes produced by stalks A and B of POJ 2878, adults and different instars of nymphs of *Perkinsiella vastatrix,* portions of midribs and leaf sheaths with punctures containing eggs, an aspirator for transferring the leaf hoppers, and insect-proof chambers. The materials and equipment used and the methods of preparing one-node cuttings for securing experimental and check cane shoots, the use of shoots from alternate nodes for experimental and check purposes, the transfer of the vector from the source to the experimental shoots, the treatment of the shoots after the insects have been transferred to them were exactly as described by Ocfemia[4]. The sugar cane POJ 2878 was chosen as material for all experiments because it is relatively free from mosaic-disease and this variety is readily infected with Fiji disease.

EXPERIMENTS AND RESULTS

Two experiments were conducted to determine whether or not the apparently healthy shoots arising from stalks A and B were virus-free.

Experiment 1. On June 15, 1937, cane shoots, 75 days old prepared from hill 10 (apparently healthy), of stalk A, were bagged to be used as sources of inoculum. A large number of non-viruliferous adults and nymphs of *Perkinsiella vastatrix* were confined in the bags to feed on the canes. On August 20, 1937, 17 adults of the leaf hopper (Fig. 1) were placed on each of 2 young shoots of a healthy POJ 2878 taken from outside sources. On September 6, 1937, 19 adult leaf hoppers were placed on each of 4 other young shoots of POJ 2878 cane, also from outside sources. The insects were allowed to feed on the experimental canes for about 2 weeks.

When the final observation was made at the end of 11 months, it was noted that none of the disease-free POJ 2878 canes became infected with Fiji disease.

Experiment 2. In December, 1937, cane shoots from the following cuttings were used: eight cuttings from healthy shoots of hill 1, 4 cuttings from hill 7, 4 from hill 9, and 4 from hill 10 of stalk A; 4 from hill 1, 4 from hill 2, and 4 from hill 6 of stalk B. The cane shoots from all of these cuttings were placed in one of the insect-proof cages, also, for use as sources of virus for transmission. A large number of adults and nymphs of *Perkinsiella vastatrix* were allowed to feed on them for several months.

On March 15, 1938, 6 young healthy shoots of POJ 2878 sugar cane were placed in the insect-proof chamber, along with the cane shoots from stalk A and stalk B. *Perkinsiella vastatrix* could feed on the canes from March 15, 1938, to June 20, 1938.

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[4] See footnote 2.

The result of this experiment was likewise negative, showing that the apparently disease-free canes that came from the original stalk A and stalk B did not carry any virus.

FIG. 1. Adults of *Perkinsiella vastatrix* feeding on leaves of young sugar cane shoots. Slightly reduced. Photographed by the photographic division, department of soils, College of Agriculture, March 10, 1938.

The susceptibility to infection with Fiji disease of apparently healthy plants from stalk A and stalk B was determined.

Experiment 1. On June 14, 1937, 5 shoots from hill 10 (apparently healthy) of stalk A bearing the node numbers 1, 2, 3, 4, and 5 were placed in one of the insect-proof cages where leaf hoppers were kept continuously in culture on Fiji-infected canes. All of the 5 shoots were kept in the cage for 15 days, and then they were placed outside for observation.

In this experiment shoot 2 was infected after an incubation period of 29 days; shoots 1 and 3 were infected after an incubation period of 36 days, and shoots 4 and 5 were infected after an incubation period of 50 days.

Experiment 2. On December 3, 1937, 5 adult viruliferous *Perkinsiella vastatrix* were allowed to feed on each of 45 shoots taken from hills 1, 7, and 10, apparently healthy, of stalk A. After an incubation period ranging from 31 to 56 days, 9 shoots, or 20 per cent, became infected, while the remaining 36 shoots showed no infection.

Experiment 3. On December 11, 1937, 10 adult viruliferous *Perkinsiella vastatrix* were allowed to feed on each of 30 shoots from hills 1, 7, 9, and 10 taken from stalk A. Of these 30 shoots, 14, or 46.67 per cent, became infected after an incubation period ranging from 31 to 56 days, and 16 did not get the disease.

Experiment 4. During the period from December 1 to 8, 1937, 3 adult viruliferous *Perkinsiella vastatrix* were placed on each of 35 shoots of POJ 2878 sugar cane obtained from outside sources. Of the 35 canes used, 9 shoots, or 25.71 per cent, became infected after a period of incubation ranging from 37 to 55 days, and 24 were not infected.

The results of these 4 experiments showed that the healthy canes that came from stalks A and B of POJ 2878 sugar cane used in experiments 1, 2, and 3, were as readily infected with Fiji disease as the canes taken from outside sources and used in experiment 4. The freedom of the canes from visible symptoms of Fiji disease did not indicate that they were either immune from or resistant to the disease.

Transmission of the Virus by Nymphs of Different Instars

In a note in *The Philippine Agriculturist* of October, 1932 (p. 358), and in the article cited in the foregoing,[5] the senior writer reported the transmission of the Fiji disease of sugar cane by adults of *Perkinsiella vastatrix.* Mungomery and Bell[6] report that in Australia they effected transmission of Fiji disease with nymphs of *P. saccharicida* Kirk. L. O. Kunkel, of the Rockefeller Institute for Medical Research, regards Fiji disease of sugar cane as an excellent example of a virosis that is biologically carried by 2 different, though related, species of insect. Owing to this fact, the adults of *P. saccharicida* and nymphs of *P. vastatrix* should be able to transmit the virus. In order to determine whether or not Fiji disease can be transmitted also by the nymphs of *P. vastatrix*, experiments were conducted to determine what stage, or stages, of the life cycle of this leaf hopper can carry the causative virus from infected to healthy canes in the Philippines.

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[5] See footnote 2.

[6] Mungomery, R. W., and A. F. Bell. Fiji disease of sugar cane and its transmission. Queensland Bur. Sug. Exp. Stat., Div. Path. Bull. 4: 5–28. 1933.

In experiments to determine the stage, or stages, of the life history of *Perkinsiella vastatrix* capable of transmitting Fiji disease, the shoots of the POJ 2878 canes used varied from 1 to 1½ months of age. The stages of the nymphs[7] used were 1st, 2nd, 3rd, 4th, and 5th instars. The insects were allowed to feed on the experimental shoots for 10 days. The results of experiments involving more than 132 experimental cane shoots and an equal number of checks may be summarized as follows: (*a*) The first instar nymphs cannot transmit the virus of Fiji disease. (*b*) The transmission of the virus can be effected by 2nd, 3rd, 4th, and 5th instar nymphs. (*c*) There was a greater percentage of transmission when 5th instar nymphs were used than when younger ones were employed. (*d*) The incubation period of the disease was shorter when 5th instar nymphs were used.

Failure of Transmission by the Young that Hatch from Eggs of Viruliferous Leaf Hoppers

Thirteen experiments were conducted by Carlos A. Calica and the writers to determine whether or not the young that hatch from eggs of viruliferous *Perkinsiella vastatrix* can transmit Fiji disease. Portions of leaf sheaths and midribs of sugar cane containing punctures that bear eggs laid by viruliferous leaf hoppers, each about 5 centimeters long, were placed between the leaf sheaths and the stalks of experimental canes. The nymphs that hatched from the eggs could feed on the shoots at will. At the end of the experiments, however, none of the plants developed Fiji disease. From these experiments the writers conclude that the Fiji-disease virus is not transmitted to the young that hatch from eggs of infective adults of *Perkinsiella vastatrix*.

The Shortest Time for Viruliferous *Perkinsiella vastatrix* to Effect Transmission

Six experiments were also conducted to determine the shortest time for viruliferous adults of *Perkinsiella vastatrix* to effect transmission of Fiji disease. The results of these experiments showed that the disease could not be transmitted when the leaf hoppers were allowed to feed for one hour, three, four, and ten hours, respectively. Transmission of the disease was effected when the infective adults were allowed to feed on the experimental shoots for at least 24 hours.

DISCUSSION OF RESULTS

Among stalks of apparently healthy POJ 2878 sugar cane selected from the field of the Department of Agronomy of the College of Agriculture at Los Baños, 2 produced from some of their eyes shoots that showed Fiji disease. It is not known why some of the nodes produced diseased shoots while the others were healthy. No attempt was made to determine the cause of the lack of uniformity of the distribution of the causative virus in the two stalks of apparently healthy POJ 2878 sugar cane.

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[7] The stages of *Perkinsiella vastatrix* used were checked for the junior writer by Mr. Arsenio Y. Coronel, of the Department of Entomology, to whom the writers are indebted.

The Fiji-disease virus was transmitted by using 50 or more 2nd instar nymphs of *Perkinsiella vastatrix* on each young shoot of POJ 2878 sugar cane. One possible reason for the failure of 1st instar nymphs to effect transmission of the virus may be the very small amount of the virus that they can carry. This supposition finds support in the fact that when older nymphs and adults are used, the incubation period of the disease is shorter than when younger ones are employed. The longer incubation period with younger nymphs seems to indicate that time is required for the multiplication of the limited amount of virus that they are capable of carrying.

SUMMARY

The results of the experiments considered in the foregoing pages may be summarized as follows: Among apparently healthy stalks of POJ 2878 sugar cane selected from a field, 2 stalks produced shoots some of which were infected with Fiji disease, and the others were disease-free.

The shoots that did not show symptoms of the disease were virus-free.

When the apparently healthy shoots that came from the 2 stalks were used in transmission experiments they became readily affected with Fiji disease.

In addition to the adults of *Perkinsiella vastatrix,* 2nd, 3rd, 4th, and 5th instar nymphs can transmit the virus of Fiji disease.

The nymphs that hatch from eggs laid by viruliferous leaf hoppers do not carry Fiji-disease virus.

Viruliferous adult *Perkinsiella vastatrix* require at least 24 hours to elapse before they can transmit the disease.

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A BUD AND TWIG BLIGHT OF AZALEAS[1] CAUSED BY SPOROCYBE AZALEAE

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(Accepted for publication December 1, 1938)

During the past five years, a bud blight has caused a constant diminution in the number of blossoms on both native and exotic azaleas growing in forests, parks, and gardens in western Massachusetts. In this area, many dead flower and leaf buds have been noted on the shrubs during the blooming period, in May. These buds bore coremia of *Sporocybe azaleae,* the

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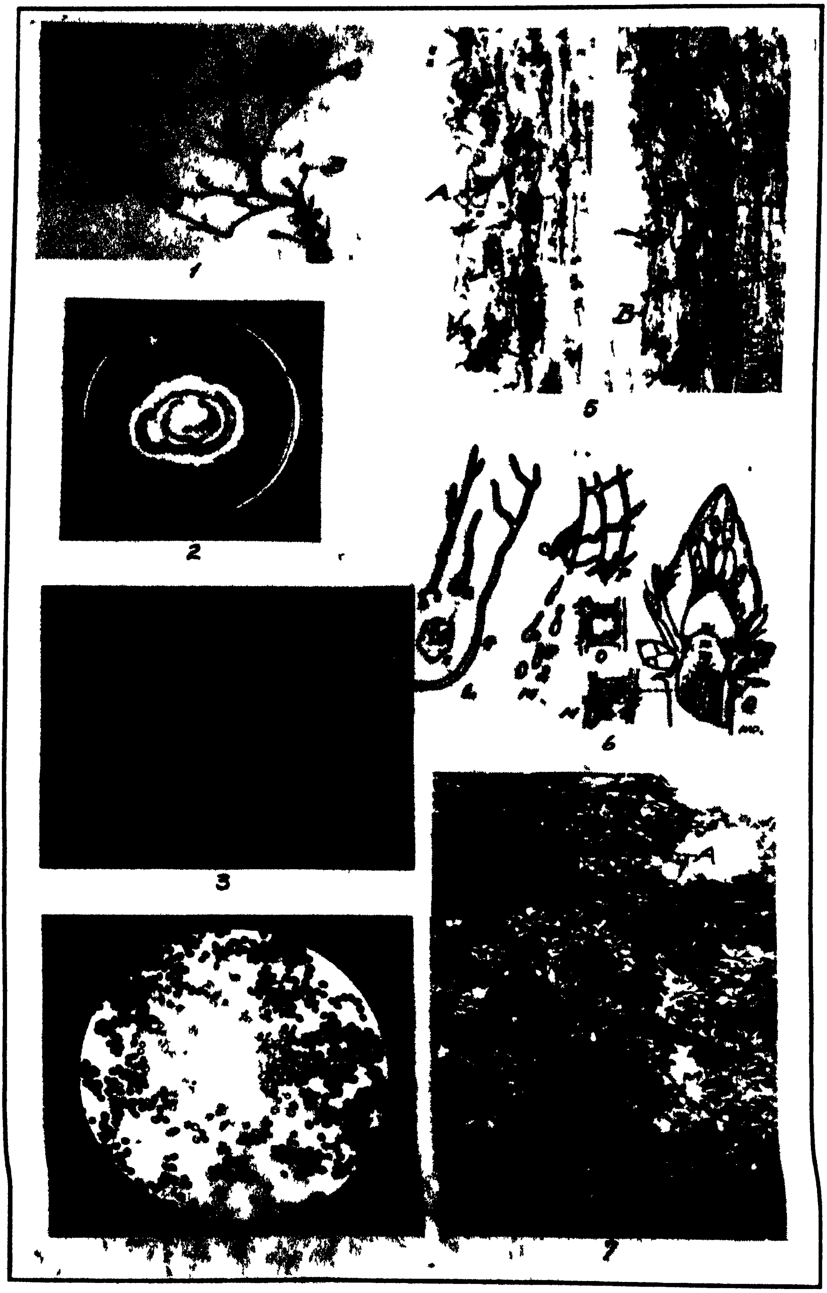
[1] The old genera *Rhododendron* and *Azalea* are here considered as one genus, *Rhododendron.* The common names azalea and rhododendron, however, have been retained as such. (A. Rehder. Manual of Cultivated Trees and Shrubs, pp. 679 and 702. 1927.)

PLATE II.



Infected parts of azaleas and the parasitic fungus, *Sporocybe azaleae*

1. Diseased twig bearing one flower, *D*, and diseased flower buds of different ages: *A*, 4 years; *B*, 3 years; *C*, 2 years, and *G*, last year.
2. A Petri dish culture showing mycelium and sporulating areas induced by a change from darkness to daylight Transferred February 14, incubated at 22° C, and photographed March 10.

causal organism, which has a fair chance of exterminating certain species of our native and cultivated azaleas unless its activities are controlled.

The fungus, *Sporocybe azaleae,* is especially interesting from a scientific, as well as an economic, point of view since it is closely related, in its classification, to *Ceratostomella* (*Graphium*) *ulmi* (Schw.) Buisman, which causes the Dutch elm disease.

A review of the available literature showed that the characteristics of the fungus in culture, its polymorphic forms and life history, had not been described. Furthermore, the symptoms, hosts, pathological anatomy, and methods of control of the disease were not well understood, so an investigation was undertaken to supply this desired information.

THE DISEASE

In 1874, Peck (3) briefly described a disease of azaleas and named the parasite *Periconia azaleae,* which Saccardo (5) afterwards described as *Sporocybe azaleae.* In 1920, Schmitz (4) reported that *S. azaleae* caused a bud rot, which was the most serious disease of *Rhododendron californicum.* Artificial inoculations made in May produced fruiting bodies of a *Sporocybe* in September. In 1931, a Sporocybe bud blast of *R. maximum* and *R. catawbiense* was reported in New Jersey. It was also reported prevalent in the humid mountainous valleys of the Southern United States.

The host indices commonly have not recorded *Sporocybe azaleae* as a parasite of azaleas in Massachusetts. However, the writer first collected the fungus in Western Massachusetts in September, 1930, and, in April, 1931, necrotic buds and twigs were observed on azaleas that had been transplanted in a rhododendron garden. Further observations showed that the disease was prevalent in other gardens in the Connecticut River Valley and the fungus was causing considerable damage.

In 1932, strict search showed that the disease was confined to azaleas in

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PLATE II.—(*Continued*)

3. A radial section of an infected azalea stem sectioned near the pith. *A.* Hyphae in the medulla. *B.–C.* Hypha extending into the wood.
4. Coremiospores showing variations in size and shape.
5. Hyphae in an azalea stem; tangential section just below an infected bud; stained and mounted in lactophenol green. *A.* Cells in a hypha. *B.* Tracheid and hypha.
6. *Q.* Longisection through the tip of an infected twig and its buds during April: T–1, tylotic area, prevents the movements of liquids into the terminal flower buds and the lateral vegetative buds, thereby causing necrosis; T–2, 3, this area also may show tyloses; T–4, penetration point, between the flower and the vegetative bud; entrance point. *L.* Coremiospore germinating in water after incubating for 12 hours: 1, emerging germtube, exosporium surrounded by a slime capsule; 2, incubated for 24 hours, slime capsule dissolved; 3, germ-tube branches after incubating for 56 hours; 4, hypha at the margin of a rapidly growing culture (length of cells 40, 72, 10, 55, 50, and 54 microns, respectively). *M.* Budding conidia from a salmon-color, plechtenchymatic tissue in culture. *N.* Sectioned healthy host pith cell, 30 microns wide, showing pits, thick walls and stored starch grains (in winter condition). *O.* Diseased pith cell removed from infected tissue; its walls are lined with gum and the pits plugged with tyloses. *P.* Infected cells removed from meristematic bud tissue during April; the viable hyphae are steel gray.
7. An infected azalea plant. *A.* Flower buds were killed and very few leaves were present. *B.* Twig entirely killed by *Sporocybe azaleae.* This plant bore no leaves during the following year and was removed.

parks and gardens, since no native azaleas growing in Massachusetts were found infected with *Sporocybe*. Following the severe winter of 1933–34, a few diseased specimens were collected on the margins of 3 forest swamps located near Leverett, Lake Wyola, and North Sugarloaf Mountain. Two of these stations were east and one was west of the Connecticut River Valley. However, in the autumn of 1935, 50 per cent of the azalea buds examined in the forests were dead, and, in November, bore coremiospores of a *Sporocybe*. This resulted in a striking diminution of the blooms during the spring of 1936. Not only flower buds, but leaf buds and twigs were necrotic.

A survey showed that the fungus was disseminated by the distribution of diseased nursery stock and the transportation of infected wild plants from the woodland and by transplanting them in the midst of healthy azaleas.

On the azaleas under observation, the terminal flower buds were first infected and experiments showed this occurred during July and August. Afterwards, lateral leaf buds and stems were attacked, so that during the following year these buds bore neither flowers nor leaves and stems. After this process was repeated for 3 or 4 years, the whole shrub died. When a twig was once infected, flower and leaf buds did not form blooms, but the leafy shoots became necrotic and generally remained intact for 3 or more years during which the fungus formed viable spores (Plate II, 1, 7).

Coremiospores generally did not appear on new buds until the buds had overwintered on the plants, and passed the flowering period, which was during the months of June, July, and August. Coremiospores, however, could be found on old, diseased buds during each month of the year. Diseased buds persist on hosts and bear viable coremiospores for 3 successive years. Even the severe winter of 1933–34, with a minimum temperature of − 26° F., did not prevent the fungus from producing viable coremiospores the following spring.

The economic aspects of this disease in azaleas are variable because they are determined by the environmental conditions and culture of the host, together with the variety under consideration. In some plantings, 60 per cent of the shrubs apparently were killed by it. In others, the greatest injury occurred to the flower buds, 98 per cent of which failed to form blooms. Furthermore, during the following year 9 per cent of the twigs also were killed. In one park, an irregular planting averaging 5 × 20 rods was so infected during the second year that 20 per cent of the flower buds failed to form blooms. The attendant believed this was because of winterkilling, until cultures from 50 necrotic buds showed 48 infected with *Sporocybe*, and later, numerous coremiospores formed on the necrotic buds. During the past 5 years, the disease has killed 30 per cent of the susceptible varieties under observation. Furthermore, the fungus has spread alarmingly among the native swamp pinks, *Rhododendron nudiflorum*.

Reports list *Sporocybe azaleae* as a parasite of both azaleas and rhododendrons. The writer, however, has collected, in Massachusetts, only 2 rhododendron buds infected with this fungus. The injuries prevalent in New Jersey (1) on *R. catawbiense* and *R. maximum* are not now present in Massachusetts.

During the past 5 years, the author has made more than 800 implants of necrotic tissues from azaleas and rhododendrons on agars. This material was collected in New Hampshire, Massachusetts, Connecticut, New York, Ohio, and North Carolina. Collections were made in botanical gardens and parks from native plants and many species of azalea and rhododendron inoculated with *Sporocybe* cultures isolated from the collections.

Sporocybe azaleae has been observed on the buds and cultured from the tissues of the following azalea species:

Rhododendron arborescens Torr. (1 plant), *R. nudiflorum* Torr., *R. canescens* Torr., *R. viscosum* Torr.

The disease was not observed on the following species which grew in proximity to diseased azaleas:

Rhododendron arborescens Torr., *R. calendulaceum* Torr., *R. californicum* Hood, *R. catawbiense* Michx., *R. dahuricum* L., *R. japonicum* Swingar, *R. lapponicum* Wahlenb., *R. maximum* L., *R. molle* G Don, *R. roseum* Rehd., *R. vaseyi* Gray.

This disease has been known by several common names. Schmitz (4) called it bud rot of rhododendrons, but later, it was reported from New Jersey (1) as Sporocybe bud blast of rhododendrons. It has been listed also as bud blight of azaleas. Bud rot is inappropriate for this disease, since the buds are seldom if ever rotted. Sporocybe bud blast only partly describes the disease, for twigs also are affected. Sporocybe bud and twig blight of azaleas is preferred by the writer for Massachusetts conditions.

The first external symptoms of the disease in flower and leaf buds appear in July and August following the initial, spring infection. Then, some of the flower buds become dwarfed to half size, turn a light brown, and shrivel, so that the tips of the basal bud scales abnormally project outward and produce a rosetted appearance.

In April and May, floral buds, infected during the previous year, shriveled slightly and the scales turned from a brown to a silvery gray. In the late autumn or during the following spring, coremia or fruiting bodies formed on the scales of infected buds, on leaf scars and bark. In the autumn, coremia appeared as small, gray, hairlike stalks or stipes about 1 mm. in height and bore at the apices gray, knoblike structures or heads composed of gray coremiospores. Later, coremia turned a dark brown and often gave the buds a spiny appearance (Fig. 1).

Old diseased buds remained attached to the azalea twigs for 3 or more years, appearing dry and firm, but never soft and rotten. These are in direct contrast with the large, plump, chestnut brown, glabrous, healthy flower buds which are one-third larger in diameter.

Ninety-eight per cent of the flower buds with these symptoms did not form blooms. However, several infected buds partly opened, showing small, abortive floral parts.

Leaf buds were less susceptible to infection than floral buds; although some healthy ones became infected each year and, failing to produce foliage, were followed by death of the twigs. In 1932 there was evidence that hyphae advanced from flower buds into the bark and the leaf buds of *Rhododendron viscosum* showed injury during the year. Yet, the severity of bud and twig infections varied with the environmental conditions, for, during the hot dry summer of 1933, inoculations produced only flower-bud infection.

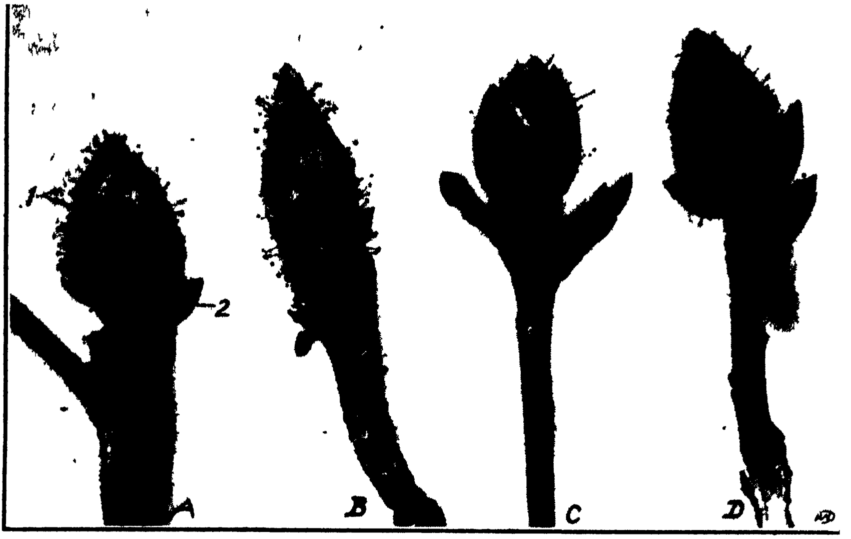

FIG. 1. Infected flower buds of *Rhododendron* (*Azalea*) *nudiflora* bearing coremia of *Sporocybe azaleae*, June 1, 1936. A. Necrotic flower bud infected during 1934: 1, coremia; 2, infected stem and leaf bud. B. Similar to A but bearing coremia during the fourth year after infection. C. Terminal flower bud infected during 1935. The other buds were infected and bore coremia which were just forming. D. Both the terminal flower bud and the lateral stem and leaf bud bearing coremia which show infection. × 5.

Dead branches, bearing necrotic terminal flower buds surrounded by necrotic twigs, gave evidence of Sporocybe infection, even though coremia were absent, because the fungus could generally be cultured from their tissues. In addition, the fungus sometimes reduces the floral parts to a black mass composed of hyphae and coremiospores intermingled with floral parts. Thus, dead twigs and buds bearing coremia may be taken as a positive symptom of Sporocybe infection.

For field diagnosis, stems bearing flower and twig buds were split lengthwise, but for microscopic examination, thin free-hand sections were made, stained with lactophenolgrün for periods varying from 15 minutes to 2 weeks, then washed and mounted in clear lactophenol.

A healthy azalea twig generally terminated with a flower bud composed of bud scales infolding immature flowers and bracts. These are outgrowths from a meristematic cone of parenchymatous stem tissue. However, the two lower bud scales differ from the others, since they bear trichomes (hairs) projecting from their inner surfaces. Beneath the layer of trichomes is a mesophyll bordering a central vascular layer containing numerous cell inclusions, while the outer layer is composed of narrow, long, cells with thick-walls, similar to other bud scales. These bud scales will be termed *coleophylla* in contrast to the bud scales, most of which press firmly to the bud and are without the layer of trichomes (Pl. II, 6).

The center of the young stem is occupied by pith, the cells of which are thick-walled, profusely pitted, and, during the dormant season, contain an abundance of stored starch grains (Pl. II, 6 N). Longisections of infected twigs showed a partial destruction of the thin-walled cells at the junction of bud scales and flowers with the parent twig and an abundance of tyloses. At this place, the flower buds were easily broken from the stem when slight force was applied. Tylosis caused brown belts one of which often separates necrotic tissues from healthy. However, in twigs bearing flower buds of long infection, tyloses gave the interior tissues a buckskin color that may extend down the stem to a branch. From here, the discoloration, which was a reliable symptom, often advanced further downward during the next growing season (Pl. II, 6 O, Q).

CULTURING THE PARASITE

Sporocybe azaleae was isolated from diseased azaleas and placed in culture by the following methods: Spore dilution; removal of hyphal tips and whole thallus; spore heads; monosporic isolations of coremiospores (Pl. II, 4), cephalospores, penicilloid spores, chlamydospores and sporidial buds. Implants of the following tissues on suitable agar also were employed: sections of coremia; sclerotia; mycelium removed from the coleophylla and other bud scales; sections of meristematic tissues from buds, pith, wood and bark. In diagnostic studies, sections from roots, stems, buds and leaves were employed.

Twenty substrata were employed for culturing this fungus. They consisted of agars, decoctions, together with both steamed and raw plant parts of seeds, flowers, buds, and stems, including those from azaleas and rhododendrons. Potato-dextrose and malt agars were most favorable for thallic growth and sporulation. However, the fungus grew and sporulated on all the raw and cooked parts of azalea and rhododendron stems and fruits; steamed filter paper; bean pods; stems of clover, alfalfa, and sweet melilot; cooked carrot and its decoctions. The growth was sparse on concentrated sugar decoctions, such as prune agar and decoctions. In general, cooked starches and cellulose could be synthesized more readily than concentrated sugars, so potato-dextrose agar was employed for thallic growth and sporulation. However, the cephalosporic and coremial stages were most conveniently cultured on steamed filter paper.

For determining the most favorable acid reaction of the substratum, Petri dishes of potato-dextrose agar were adjusted to pH values varying by gradients of 0.5 from 3.5 to 8.5. These cultures were incubated at 20° C.

for 60 days and observations showed the best pH value of this substratum for procuring different types of growth:

1. Surface of the agar in the Petri dishes was covered with mycelium:
    a. First at 7.02 after 30 days; best for thallic growth.
    b. Last at 4.05 to 8.0; covered after 50 days.
2. First conidia observed at 6.08 after 11 days.
3. Sporulation, 5.0.
4. Limits for acidity, above 3.5 or about 3.0.
5. Limits for alkalinity, above 8.5 or about 9.0.
6. Growth differs only slightly at pH 7 and 8, after incubating for 10 days; the greatest difference lies between pH 6 and 7.

As a general laboratory practice, 2 drops of sterile 28 per cent lactic acid were added to each sterile Petri dish before the melted agar registering pH 6.8 was inserted. The average radial expansion in growth of the fungus incubating on agar at 20° C. was 4 mm. weekly.

The most favorable temperature for thallic growth was determined by subjecting properly prepared cultures to centigrade degrees of temperature ranging from 0° to 30° and varying in gradations by 5°: optimum, 22–25; minimum, 8–9; maximum, 33.

An active culture incubated on potato-dextrose agar at 25° C., for one week, had a slightly raised margin, varying from a light gray to a mouse gray which bordered a wide olive-color felty, sporulating area, but when incubated at 10° C., the hyphae were raised 1 or 2 mm., forming a dark olive-green color due to the belt of conidia. Hyphae in a culture at this temperature did not penetrate the agar more than 1 mm., while at favorable temperatures, it often penetrated 2 or more mm. and generally contacted the basal glass surface. However, these results could be changed by varying the substratum and light together with incubation period and humidity (Pl. II, 2).

Sporocybe azaleae is a pleomorphic fungus, the stage of which will be described at a later date.

PATHOLOGICAL ANATOMY AND LIFE HISTORY

The initial infection occurred in meristematic tissues located about the base of the lower scales of flower buds. Rainwater washed coremiospores from infected parts to the coleophylla of newly formed buds. The hairy surfaces arrested the spores (Pl. II, 6 A) which germinated and their germ-tubes penetrated adjoining meristematic tissues, and, finally, the meristematic bud cone. From here, the hyphae advanced upwards into the tissues of flowers, bracts, parts of leaf and buds and downward most readily through the bark. However, hyphae advanced both radially and longitudinally through the stem tissues (Pl. II, 3, 5). They entered the pits in the thick-walled pith cells, removed the contents, then advanced crosswise of the stem by traversing the medullary rays with no apparent injury to the adjoining xylem. Here the hyphae advanced by dissolving the middle lamellae. Hyphae also branched within host cells, traversed the vascular system and woody tracheids and penetrated parenchymatous cells in the bark. The cambium was penetrated before tyloses blocked the advance, but hyphae sometimes penetrated between two cells while each contained tylotic gum materials. Compact hyphal masses, sclerotia, formed in cells near the surface of bark and the bud scales. These activities of the parasite killed buds, prevented the formation of new blooms, leaves, and stems; arrested photosynthesis; girdled the bark by plugging the phloem; and, under favorable conditions, caused necrosis of twigs with their buds and finally killed the whole plant.

The position of the organism in azalea twigs was determined by culture methods. Properly prepared sections from infected and from apparently healthy azalea and rhododendron twigs were implanted on potato-dextrose agar and incubated at 20° C. The implants were prepared each month during 1933 and 1935, which included a dry and a wet summer. Other tests, however, were made at irregular intervals during each month of the growing season, from April to October, inclusive, and during the years 1930, 1931, 1932, 1934, and 1936, which served as checks. The results of this culture work follow:

1. Sometimes *Sporocybe* was accompanied by other fungi in the tissues of azaleas, such as *Alternaria, Sphaeropsis, Phoma, Phomopsis, Trichoderma, Verticillium* and *Nectria.*
2. *Sporocybe azaleae* was cultured from diseased azalea twigs, and it remained viable in the host during each month of the years 1933 and 1935.
3. Seventy-four per cent of the bud scales from apparently infected buds showed *Sporocybe* present each month of the year.
4. Thirty per cent of the bark implants from twigs bearing necrotic buds produced *Sporocybe.*
5. Eight per cent of the wood implants from infected azaleas produced the fungus.
6. Thirty per cent of the pith implants consisting of tissue located below infected buds contained *Sporocybe.*
7. The fungus could be cultured from apparently infected dead twigs just as long as the inner bark remained green and intact.
8. In the bud inoculations, the fungus advanced into the stem from 3 to 5 mm. the first year and to a decimeter the second year.

INOCULATIONS

The inoculum was obtained from cultures isolated by methods previously described. Employing these cultures, preliminary tests showed that the most virulent inoculum consisted of mixed cultures that had been isolated from coremia and bark tissues; so these were generally employed. They were designated as E–31, isolated from an infected azalea stem; B–16 and G–44, from coremia on buds of *R. nudiflorum.* The prepared inoculum consisted of sterile distilled water containing one or more of the following:

Nongerminated viable conidia, germinated conidia, conidia with mycelium and mycelium alone.

Special precautions were observed so as to inoculate plant parts each month of the growing season from April to October inclusive; to inoculate both mature and immature tissues of stems, leaves, fruits, and flowers; both open and closed flower and leaf buds; to inoculate species of azaleas and rhododendrons; to make reciprocal inoculations employing species of azaleas; to care for inoculated parts and make proper tests for infection.

TABLE 1.—*Species of azaleas and rhododendrons inoculated, number of inoculations and hosts infected*

Hosts inoculated	No. of inoculations	No. of infections	Inoculation showing infection				
			Buds	Stems	Leaves	Capsules	Blossoms
Rhododendron arborescens	40	2	2	0	0	0	0
R. canescens	40	11	8	1	0	2	0
R. nudiflorum	52	18	14	1	0	2	1
R. viscosum	48	2	2	0	0	0	0
R. californicum	34	2	2				
R. catawbiense	34	1	1				
R. arborescens	8	0					
R. maximum	8	1	1				
R. molle	8	0					

Results shown in table 1, together with other observations, seemed to warrant the following statements:

1. Infection was most successful when the inoculum was placed in the axils and outer scale cups of buds. Here the spores germinated in collected water and their hyphae penetrated the near-by meristematic tissues of both flower and vegetative buds, and an annual advance of an inch was often noted.
2. Wounds in the bark afforded a favorable entrance for hyphae. In the meristematic tissues, artificial puncturing was necessary for infection. The fungus advanced 5 to 30 mm. annually.
3. The fungus penetrated woody tissues of azalea, but advanced very slowly, 1 to 5 mm. annually.
4. Inoculations performed during August and September produced the best infections.
5. Only one of the 8 inoculations of buds on *Rhododendron maximum* and *R. catawbiense* produced infection.
6. No definite external symptoms were noted in viable leaves inoculated under natural conditions. Yet, the fungus grew and sporulated on the surface of excised leaves exposed to experimental conditions.
7. Inoculated capsules showed infection and coremia. In general, the fungus grew and formed cephalospores and conidia when properly inoculated capsules were stored in damp chambers.
8. ***R. arborescens*** and ***R. viscosum*** were somewhat resistant while ***R.***

nudiflorum and *R. canescens* were susceptible to the strains of *Sporocybe* employed; the rhododendrons were considered resistant.

9. Azalea plants infected by artificial inoculation lived about 5 years, but most of them remained leafless with green bark during the fifth year following inoculation.

CONTROL MEASURES

Three types of control of azalea bud blight were investigated, *i.e.*, pruning, spraying, and dusting.[2]

All the diseased buds were pruned from highly infected plants of *Rhododendron canescens* during late autumn and early spring for two successive years. Furthermore, during each summer month that followed, all stems with diseased buds were pruned back at least one inch and burned. As a check, diseased transplants remained undisturbed. At the close of the second season, in 1936, 92 per cent of the flower buds on the controls bore blossoms, while buds failing to bloom were apparently killed by a species of *Sporocybe*. After 4 years, all the infected controls but one were necrotic and removed, while the pruned plants were among the healthiest in the garden. This method of pruning and destroying the infected buds has been practiced in the City Park at Rochester, New York, and the disease has there been successfully controlled since 1933.

A thorough drying of old infected azalea buds and woody tissue killed the contained parasite, but the spores thereon remained viable more than a year. From the above evidence it was decided that early pruning and destroying of diseased plant parts was a suitable control.

Dusts and sprays also were employed for controlling the fungus. By means of a hand duster, copper-lime dust 57 was applied to all buds before the flower buds opened in the spring, so as to kill the spores formed on the exterior of old infected buds. The surface of the buds was constantly covered with dust by dusting every 2 weeks during the growing weather in the spring until blossoming time. After blossoming, the vegetative buds were dusted until they opened. Matured leaves and newly forming buds were given monthly applications until leaf fall, when biweekly dusting continued until the advent of freezing weather. The near-by checks were not dusted.

The copper-lime dust decreased the infection of leaf buds during the 2 years it was applied. Counts showed slightly less than 8 per cent infected buds on dusted plants, while the nondusted controls bore 30 per cent. But some infected buds on both sprayed and check plants were the accumulation of a previous year. Two experiments showed 90 per cent control by dusting from September 1 to November 1, or by "fall applications."

A liquid Bordeaux (5:5:50), was applied to infected buds bearing viable conidia. The schedule of application closely followed that already described for dusting; dormant and pre-blossoming; after blossoming; monthly until leaf drop, when the buds and stems thereafter were kept constantly covered with spray until freezing weather appeared. This spray killed coremiospores and controlled the fungus better than pruning and dusting, but the inconvenience of application and undesirable appearance of the plants mitigated against its use by florists and horticulturists.

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[2] The writer was assisted in this part of the work by Prof. Wayne Lowry, who met an untimely death in an automobile accident before his part of the work was completed

Pruning and destruction of diseased twigs and buds, dusting with copper-lime dust 57, and spraying with a 5:5:50 Bordeaux will control this fungus. It is to be noted that fall applications produced 90 per cent of the control, since infection occurs during this period.

SUMMARY

This investigation was undertaken to study the life history of *Sporocybe azaleae* and the disease that it engenders in azaleas and rhododendrons.

A twig and bud blight caused by *Sporocybe* threatens to exterminate some species of the cultivated and native azaleas in Massachusetts.

The fungus and its parasitic behavior is of timely interest, since mycologists consider it closely related to *Graphium ulmi,* cause of the so-called Dutch elm disease. Like that disease, this also plugs the vessels with tyloses, and necrosis results.

This fungus has been reported parasitic on rhododendrons in New Jersey and California, but only 2 infected buds were observed on these plants in Massachusetts.

Sporocybe bud and twig blight of azaleas is the common name suggested for Massachusetts conditions of the disease.

The symptoms of diseased buds, flowers, and plants are described.

The isolated fungus grew well and sporulated on cooked materials containing starches and sugars. A pH near 6 was preferable for the substratum; the optimum temperature for growth was 22–25° C. A maturing culture is described.

The initial infection occurred in the axils of the lower scales on buds where bud and stem tissues were penetrated. From here, it advanced into the bark and pith cells filled with stored starch grains, and traversed the vascular system.

The fungus killed buds, prevented the formation of new flowers, leaves and stems, arrested photosynthesis, "girdled" the stems, and plugged the phloem, all of which resulted in necrosis.

As long as the inner bark remained green, the fungus could be cultured from infected azalea stems.

Nine species of azaleas and rhododendrons were inoculated and the results showed ***Rhododendron arborescens*** and ***R. viscosum,*** resistant; ***R. nudiflorum*** and ***R. canescens,*** susceptible. Rhododendrons were considered resistant.

Pruning and destroying diseased materials, and either spraying or dusting with a form of Bordeaux controlled the disease.

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SCOUTING AND SAMPLING ELMS WITH SYMPTOMS COMMONLY ASSOCIATED WITH THE DUTCH ELM DISEASE AS AN AID IN ERADICATING CERATOSTOMELLA ULMI

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INTRODUCTION

The purpose of this paper is to present the results of a study of the method of scouting and sampling trees with symptoms commonly associated with the Dutch elm disease as an aid in eradicating *Ceratostomella ulmi,* the causative organism.

Symptom sampling for the Dutch elm disease consists of: (a) scouting for trees exhibiting the external symptoms commonly associated with the disease; (b) cutting off twigs or branches showing these external symptoms and examining them for the internal symptom—a brown discoloration in the xylem; (c) collecting samples from those having internal symptoms associated with the external symptoms.

Neither external nor internal symptoms of the Dutch elm disease appear to be specific; and the external symptoms in particular are extremely variable (3, 4, 6). The latter may include dwarfed, curled, or normal leaves that may show wilting, yellowing, drying, or browning. Although wilted leaves and those showing yellowing may return to a normal appearance, most leaves that show symptoms fall prematurely and the defoliated branches may either eventually refoliate or die. Wilting probably is the most frequent initial symptom in the spring and early summer, while yellowing and browning of the leaves are more often the first expressions of the disease in trees that are not visibly affected until later in the season. The prevalence of the different types of symptoms may vary from year to year, perhaps in response to varying weather conditions. Succulent twigs killed suddenly often show a curling at the tips. External symptoms may spread rapidly throughout the crown or remain localized. Internal symp-

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[1] The writers wish to thank Curtis May for his advice and helpful criticism throughout the investigation and in the preparation of the manuscript. They wish to acknowledge the aid of Paul V. Mook, Agent, Division of Forest Pathology, Bureau of Plant Industry, and of J. William Pike, formerly Agent, Bureau of Entomology and Plant Quarantine, in collecting and compiling portions of the data, and to thank K. A. Layton, H. C. Miller, and H. E. Battersby, also of the Bureau of Entomology and Plant Quarantine, for their aid in establishing plots of series I.

toms consist of the development of tyloses and a browning of affected xylem, the discoloration being similar in appearance to that produced by other causes.

The nonspecificity of the symptoms and their similarity to those associated with diseases of elm caused by other vascular wilt fungi, notably *Dothiorella ulmi* Verrall and May (8), earlier referred to as the elm *Cephalosporium* (1, 2), and a species of *Verticillium* (5), necessitate culturing samples from suspected trees to determine whether *Ceratostomella ulmi* is present.

To study symptom development in its relation to the problem of symptom sampling, investigations were begun in 1936 and further expanded in 1937.

METHODS

Three series of plots were established in New Jersey. Series I, established in the spring of 1936, consisted of 5 plots, located, respectively, in Montclair and Maplewood, Essex County; near Princeton, Mercer County; Somerville, Somerset County; and Chatham, Morris County. These plots varied in size and were selected to include urban and rural elms of high landscape value, as well as natural stands on well-drained river terraces and on poorly drained areas. All elms, 2 or more inches in diameter at breast height, which were within the selected areas, were considered as plot trees. Their number varied from 220 to 469 per plot. In all, 1,514 trees, largely *Ulmus americana* L., were included in this series of plots.

Series II, also established in the spring of 1936, consisted of 725 American elms in 29 plots, each containing 25 trees. Most of these plots were in Morris County near Morristown, Wharton, Lincoln Park and Chatham, one was east and one southeast of Ridgewood in Bergen County, and one was near West Caldwell in Essex County. These plots were selected in areas in which numerous cases of Dutch elm disease had been found in 1934 and 1935. In many of these plots the trees were so selected as to include several with 10 per cent or more of the crown dead, but only a few were as much as half dead at the time this study was initiated.

Series III consisted of 21 plots established in the spring of 1937 in conjunction with an elm population survey of Sussex County.[2] The plots were 2 chains wide and extended 1½ miles north from scattered points within the county, all simultaneously determined by the arbitrary selection of a single point. Observations were made of all elms 2 or more inches in diameter at breast height and some approaching that size. While most of the 2,185 trees in these plots were *Ulmus americana,* some trees of *U. fulva* Michx. were included.

Plots of series I and II were observed for external symptoms at approximately 2-week intervals during the summers of 1936 and 1937, and those of series III during the summer of 1937. In series I and II observations were begun in early June in most plots in 1936 and in all in 1937. They did not begin until the latter part of June, 1937, for series III. They continued in I and II until October 1, 1936, and the latter part of September, 1937. In series III the observations were terminated on September 15, 1937.

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[2] Fate, L. R. An estimate of the population, size, and vitality of elm trees in Sussex County, New Jersey. Unpublished office report of the Bureau of Entomology and Plant Quarantine and the Bureau of Plant Industry, U. S. Department of Agriculture.

In the course of these observations, the following symptoms were considered as possibly associated with the Dutch elm disease: wilting and discoloration of otherwise normal leaves, even if those affected were limited to very small twigs; dwarfed or curled leaves appearing locally in the crown; sharply localized defoliation; and dead twigs with buds still present, indicating recent death. None of these, however, was considered a symptom if limited to broken twigs or branches; nor were the generally scattered yellow leaves that appeared in late August and September so considered.

Accurate diagrams showing the location of symptoms were made of all trees in plots of series I and II at each observation period in 1936 and 1937. The nature of the symptoms was recorded in supplementary field notes. In late summer and early autumn, 1936, using the diagrams and notes, as well as any symptoms then present, as guides, from 2 to 20 specimens were collected from each tree in series I that had shown external symptoms at one or more observation periods. The specimens which upon examination showed internal discoloration were cultured to determine whether *Ceratostomella ulmi* was present. Similar sampling of trees in plots of series II was carried on during 1936 and 1937, but lack of time prevented as thorough sampling. Some symptoms not especially suggestive of vascular wilts were not sampled. Sampling in plots of series I and III in 1937 was limited to symptoms most suggestive of vascular wilts.

Four hundred nineteen trees of series II were felled during the winter of 1937–1938 and were thoroughly examined for internal symptoms of disease. As the trees were felled, their trunks were cut into segments varying from 4 to 8 feet in length and the junctions of all branches larger than an inch in diameter, as well as some smaller, were severed and the cuts carefully examined for discoloration. The discolorations found were traced to their limits, diagrams were drawn in the field to show the extent of each discoloration, and notes were taken recording the annual ring in which each discoloration appeared. Samples for culturing were collected from near the limits of the discolorations. Superimposing discoloration diagrams upon those made previously, and showing the extent of external symptoms, indicated whether external symptoms had been present on the twigs or branches having internal discoloration. The year of infection and of subsequent invasion of new growth sheaths was determined by the ages of the discolored annual rings. Combining data on ages of discolored annual rings with the superimposed external and internal symptom diagrams made it possible to determine, for the years 1936 and 1937, whether the external symptoms were associated simultaneously with the internal discoloration for those years. From this it could be determined whether trees, first infected in

1936 or 1937, showed external symptoms on the infected branches in the year of their infection, and whether trees previously infected showed such symptoms during 1936 and 1937.

Specimens showing both external and internal symptoms were collected from all plots and cultured as follows: chips were placed in Petri-dishes containing potato-sucrose agar and any mycelium developing subsequently was diagnosed microscopically. Specimens collected from the felled trees of series II were also cultured by a modification of Walter's technique (9). The chips were treated with hydrogen peroxide and introduced into sterile Petri-dish moist chambers, where they were kept moist for at least 3 weeks at a temperature of approximately 60° F. If, during this period, coremial fructifications appeared, some of the spore-containing exudate from the coremial heads was transferred to potato-sucrose agar for identification, since organisms closely related to *Ceratostomella ulmi* form somewhat similar coremia. The second method was employed because, under some conditions, it has been found more efficient than the first. Both methods, used together, have been found more effective than either used alone.

OBSERVED PREVALENCE OF SYMPTOMS COMMONLY ASSOCIATED WITH THE DUTCH ELM DISEASE

Results of studies carried on during 1936, based on plots of series I and II, showed that as the season progressed there was a pronounced increase in the number of trees showing symptoms commonly associated with, but not specific for, the Dutch elm disease. This tendency reached its peak about September 15. Thereafter, presumably because of general autumnal defoli-

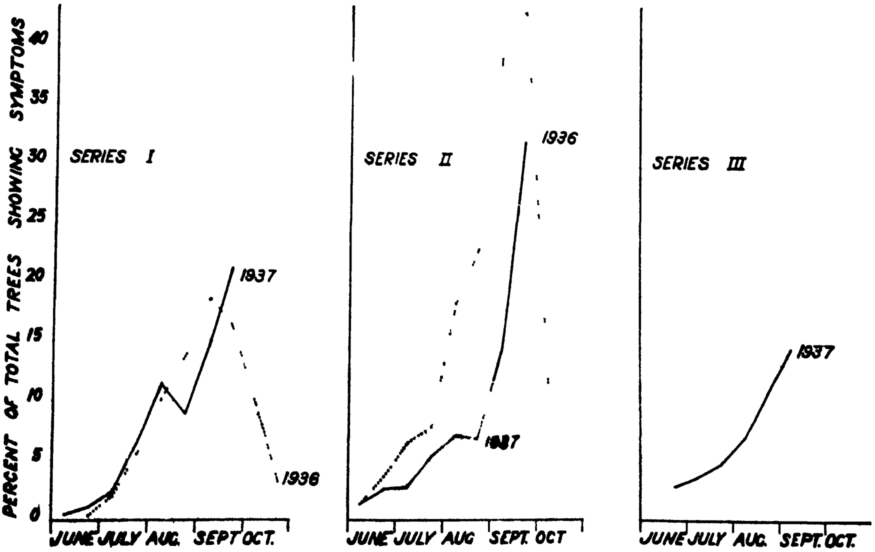

FIG. 1. Prevalence of symptoms commonly associated with the Dutch elm disease in study plots observed biweekly during 1936 and 1937.

ation, the number of trees showing external symptoms decreased. Probably early autumnal yellowing of leaves and defoliation, occurring locally within the trees, accounted for much of the increase in symptoms during late August and early September.

These results together with similar data obtained on the prevalence of symptoms in plots observed in 1937 are shown in figure 1. Both 1936 and 1937 data indicate that more symptoms appeared in elms late in the summer than were present during June, July, or the early part of August. Very few of the trees showing symptoms, however, proved to be infected with *Ceratostomella ulmi*.

As seen from table 1, the ratio of the number of new symptom trees with the Dutch elm disease to the total number of new symptom trees was highest during the latter half of June and decreased thereafter. There was a corresponding increase in the number of new symptom trees sampled for each new tree found affected by the Dutch elm disease.

TABLE 1.—*Biweekly increases in the number of plot trees showing symptoms in 1936, the numbers of these new symptom trees found affected by Ceratostomella ulmi and their numerical relationships*

Observation period	Increase in the number of plot trees showing symptoms	Number of new symptom trees infected with *C. ulmi*	Ratio of the number of new symptom trees with the Dutch elm disease to the total number of new symptom trees	Number of new symptom trees sampled to find one new tree with the Dutch elm disease
June 1–June 15				
June 16–June 30	18	6	0.33	3.0
July 1–July 15	28	5	0.18	5.6
July 16–July 31	78	4	0.05	19.5
Aug. 1–Aug. 15	136	3	0.02	45.3
Aug. 16–Aug. 31	86	0	0	
Sept. 1–Sept. 15	173	0	0	

Not all trees showing external symptoms had associated xylem discoloration. Figures obtained for trees in plots of series I in 1936 and trees in the most thoroughly sampled plots of series II in 1936 and 1937 are included in table 2 and indicate that from ⅛ to ¼ of the trees observed showed some

TABLE 2.—*Percentage of plot trees observed showing symptoms prior to September 1 and percentage of those infected as found by symptom sampling*

Series	Year	Number of trees observed	Percentage with external symptoms	Percentage in which internal symptoms were found associated with external symptoms	Percentage infected as found by symptom sampling
I	1936	1514	21	10	0.007
II	1936	387	26	8	2.325
II	1937	387	12	3	0.256

TABLE 3.—*Summarized case histories for trees found infected with Ceratostomella ulmi that did not show symptoms before September 1*

Tree no.	D.B.H. (in.)	First year diseased[a]	Symptoms shown		Detectable from symptoms[b]	Period diseased (years)[c]	Period recovered (years)[d]	Infections		
			1936	1937				No.	Annual ring	Extent (feet)
1	15	1937	No	Yes	9/22/37	1		1	1937	4
2	6 and 8	1934	Yes	No	No	1	3	1	1934	twigs
3	13.5	1937	No	Yes	No?	1		1	1937	2½
4	6 and 8	1936	Yes	No	10/6/36	1	1	1	1936	6
5	16	1935	Yes	Yes	No	1	2	1	1935	6
6	8 and 10	1933	Yes	No	No	2	3	1	1933 1934	25
7	8 and 11	1931	Yes	No	No	4	3	3?	1931 1932	16
									1932 1933 1934	18
									1931 1932 1933	22
8	15	1935	Yes	Yes	9/22/37	3		4?	1935 1936	5
									1937	8
									1937	15
									1937	45
9	7	1932	No	No	No	1	5	1	1932	6
10	5	1926	No	No	No	1	11	1	1926	10
11	6 and 8	1936	Yes	No	10/5/36	1	1	1	1936	4
12	3 and 6	1934	No	Yes	9/23/36	1	3	1	1934	6

[a] The earliest discolored annual ring from which *C. ulmi* was isolated.

[b] Infections in parts of the tree that had shown external symptoms were considered detectable when the symptoms were first observed. Infections in trees or parts of trees that did not show symptoms were considered not detectable.

[c] The discolored annual rings from which *C. ulmi* was isolated were considered to indicate the years in which the current growth sheath had been infected. Discolorations limited to the base of the trunk were not included, as they may not signify years in which the tree has been infected in the current growth sheath.

[d] The number of healthy rings laid down over the outermost infected ring was assumed to indicate the number of years since the tree had had infection in a current annual ring. Such trees were considered to have "recovered" from the disease, though *C. ulmi* was still viable within them.

external symptoms at some observation period prior to September 1 and had to be sampled in the field and examined for internal discoloration. Internal discoloration was found associated with the external symptom in ⅓ to ½ of the trees sampled; *i.e.*, in 3 to 10 per cent of the total trees observed. Relatively low percentages of the specimens collected from trees showing both external and internal symptoms gave *Ceratostomella ulmi* when cultured.

THE OCCURRENCE OF CERATOSTOMELLA ULMI IN PLOT ELMS IN THE PRESENCE AND IN THE ABSENCE OF EXTERNAL SYMPTOMS

During the winter 1938–39, 419 elms were removed from 20 of the plots of series II. Of the other 81 trees, originally included in these plots, 10 had previously been found by symptom sampling affected by the Dutch elm disease. Those 10 trees were eradicated; the rest had been felled by the owners or removed by mistake.

With the felling and careful examination of the 419 remaining elms for internal symptoms and the culturing of all discolored wood, 14 trees were found infected by *Ceratostomella ulmi* in addition to the 10 previously found by symptom sampling. Two of the 14 had shown symptoms induced by *Ceratostomella ulmi* before September 1; one in 1936 and the other in 1937. These particular symptoms, however, had not been sampled because of the greater prominence of other symptoms not induced by the Dutch elm disease fungus. Thus, of the 24 trees infected by *C. ulmi,* half had shown symptoms that might have been sampled by a scout before September 1, in either 1936 or 1937.

The 12 non-symptomatic cases of *Ceratostomella ulmi* infection were not confined to elms that might be eliminated in a sanitation program involving the removal of trees whose crowns were 50 per cent or more dead, since the crown of none of the trees was more than 10 per cent dead in 1936. Although several showed a considerable increase in dead wood in 1937, no appreciable quantity of the dead wood was adjacent to the infections, and the greater part of this dying was attributed to other causes. Neither suckering, late opening of the leaf buds, nor early entry into the winter condition would have served as indicators of these hidden infections.

The case-history information available for the 12 trees that, though infected, failed to show symptoms of disease before September 1, is given in table 3. Four of the 12 trees were first infected in 1936 or 1937 and showed no symptom prior to September 1 in the season of their infection. The remaining 8 trees, infected before 1936, showed no symptoms in association with the *Ceratostomella ulmi* infections prior to September 1, 1936 or 1937. Five of these trees did show external symptoms after September 1 that later proved to have been present on limbs or twigs infected by the fungus and might have been found to be infected had intense scouting been carried on at that time; but, because of the abundance of autumnal symptoms, such scouting was then impractical. Nine of the twelve trees had infections in non-current annual rings, which had become occluded by the failure of *C.*

ulmi to grow centrifugally and infect the new growth sheath. The occlusion of infections resulted in the recovery of these trees in the sense that they did not subsequently show symptoms due to the infection, a condition previously noted by Smucker (7) and Walter (10) for small American elms; the fungus was still viable, however, in an 11-year-old infection of this type and in others more recent. Infections unaccompanied by symptoms were mostly localized, a few extended for more than 10 feet, and one 45 feet long was found.

SUMMARY

Observations, made every 2 weeks on 2,239 trees in 2 series of plots during 1936 and on 4,424 trees in 3 series of plots during 1937, indicated that as the summer season advanced, the number of elms showing external symptoms commonly associated with the Dutch elm disease increased markedly until the middle of September, when defoliation set in.

Only a small percentage of the trees observed was actually infected with *Ceratostomella ulmi.* The infected trees that showed symptoms conjoined with *C. ulmi* infection before September 1, nearly all did so before the middle of August.

The efficiency of symptom sampling was at its maximum during the latter part of June and throughout July.

From 12 to 26 per cent of the trees in the most completely sampled plot series showed external symptoms and had to be examined in the field for sapwood discoloration. From 3 to 10 per cent of all the trees in these plots had internal symptoms present in those branches or twigs that showed external symptoms. They, therefore, had to be diagnosed by culturing collected samples. From 0.007 to 2.325 per cent of all the trees were, by culturing, found infected.

In one carefully scouted and sampled plot series, 12 trees showing symptoms were found affected by the Dutch elm disease. Twelve others, which had not shown symptoms prior to September 1 in either 1936 or 1937, were found infected with *Ceratostomella ulmi* by careful examination of 419 felled plot trees. Of the 12 not showing symptoms before September, 4 were first infected in 1936 or 1937 and showed no symptoms prior to September 1 in the season of their infection and 8, first infected before 1936, also showed no symptoms before September 1 in 1936 and 1937.

Nine of the 24 trees infected with *Ceratostomella ulmi* recovered from the disease in the sense that the infection became occluded, though the fungus was found to have remained alive within certain of the trees for several years. The ultimate result of the presence of such infections in areas from which the disease is to be eradicated is at present a matter of conjecture and worthy of serious consideration.

DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY, IN COOPERATION WITH
BUREAU OF ENTOMOLOGY AND PLANT QUARANTINE AND
CIVILIAN CONSERVATION CORPS.

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AVOCADO SUN-BLOTCH IN FLORIDA

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(Accepted for publication February 11, 1939)

This disease has been known in California for a number of years, where it is widely distributed in the avocado-producing sections of the State. It is recognized as a troublesome problem in avocado production and is considered to be a disease of virus origin. Characteristic symptoms occur on leaves and fruits, and the branches are affected in size and habit of growth. Badly affected trees are dwarfed and may exhibit a drooping or willowy type of growth. While the natural mode of dissemination of the disease is not yet fully understood, it is chiefly spread in commercial plantings through propagation by budding and grafting. This fact has aided materially in controlling the disease and in the isolation and elimination of diseased trees as factors in spreading sun-blotch.

Definite cases of sun-blotch were observed recently in a commercial avocado planting near Avon Park, Florida. In June, 1938, the writer found a peculiar condition on leaves, twigs, and fruits of an avocado tree located in this grove. This tree was stunted and abnormal in shape and size, compared to surrounding trees in the grove. Specimens of leaves and fruits were collected at the time. Their subsequent examination showed a striking similarity between these specimens and the illustrations of sun-blotch as reported in California by Horne.[2]

The grove was visited later and a more careful examination was made of the same tree and surrounding trees. It was found that 30 trees in 2 rows across the grove were all more or less affected by the same trouble, with characteristic markings on leaves and fruits similar to those observed on the first tree a month before. Two varieties were concerned—the Nabal and Taylor. The degree of infection varied with the individual trees. Some were slightly affected, with symptoms barely noticeable except in reduced size of the tree. Others were more severely affected, the symptoms appearing chiefly on leaves and fruits. The Nabal tops appeared to be more severely affected than the Taylor tops. All trees in these 2 rows were smaller than those on either side; many of the Nabal trees were more dwarfed or stunted than the Taylor trees. It was learned that the trees in these 2 rows were originally planted to the Taft variety, budded on West Indian seedling roots. The Taft trees failed to produce satisfactorily and the tops were cut back and reworked to Nabal and Taylor varieties. No evidence of the same trouble was found at this time on any of the adjacent trees or other trees in this block, though Nabal, Taylor, and other varieties were present on either side of the 2 affected rows.

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[1] Senior Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

[2] Horne, W. T. Avocado diseases in California. Calif. Agr. Exp. Stat. (Berkeley) Bull. 585. 1934.

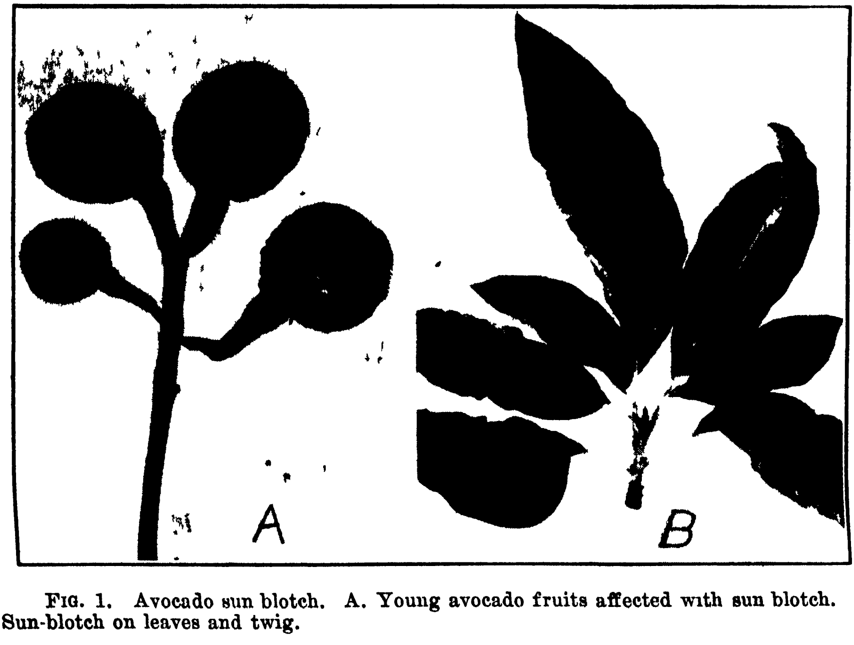

FIG. 1. Avocado sun blotch. A. Young avocado fruits affected with sun blotch. B. Sun-blotch on leaves and twig.

Affected leaves and fruits from the diseased trees were sent by the owner to Prof. W. T. Horne, in California, who identified the specimens as sun-blotch, as it is known in California. Photographs of affected leaves and fruits were later sent by the writer to Prof. Horne, and he stated they were undoubtedly representations of the disease known as sun-blotch (Fig. 1). All affected trees in the 2 rows were immediately dug up by the owner and destroyed.

Having confirmed the identity of the disease as sun-blotch, an effort was made to trace its entry, history, and distribution in Florida. In the printed literature available no mention was found of sun-blotch occurring in Florida other than a reference by Wolfe[3] of a single case observed in 1933. He, however, gave no details as to location, extent, or type of infection noted. Schroeder[4] later cited this same reference in the Yearbook of the California Avocado Association, 1935. Inquiries were addressed to the Florida State Plant Board, Gainesville, Florida, and the Mycological and Disease Survey, U. S. Department of Agriculture, Washington, D. C, to learn whether or not sun-blotch had been definitely reported as occurring in Florida previous to this time. Erdman West, mycologist of the Florida State Plant Board, replied that they had no report of its incidence in that State. Dr. H. A. Edson, U. S. Department of Agriculture, wrote that a careful search of the records of the Plant Disease Survey failed to show any report of sun-blotch of avocados in Florida.

The specimens found in June were the first observed by the writer over a period of more than 20 years of observation and study of avocado diseases within the State. That the disease did occur in sporadic and isolated cases prior to this was evident from information received by questioning various growers. Nineteen hundred and thirty-two is the first definite date reported.

An avocado grower at Homestead, Florida, reported 2 sporadic cases of sun-blotch that developed on widely separated trees in 1932 and 1933. The history is as follows: The first case noted was a single tree in the Homestead area. A West Indian seedling avocado was topworked to the Carlsbad variety, the scions having been obtained from a nurseryman of Carlsbad, California. The crop that matured on this tree in 1932 showed typical specimens resembling sun-blotch. The Carlsbad top was later removed and the stump grafted to a West Indian seedling. These grafts died, and the stump was allowed to sprout and develop into a tree, the original West Indian tree. No evidence of sun-blotch has occurred on the leaves or twigs, but the tree has not fruited since.

The second case was a single tree in a nursery several miles distant. A Collinson scion was grafted on a Mexican stock in 1928. The origin of the Collinson scion is unknown. A severe case of sun-blotch appeared on the tree in 1933 affecting the foliage and fruits. The tree was later destroyed.

The same avocado grower also reports having seen in 1933 a tree at Coconut Grove, Florida, affected with what was taken to be the same disease. The tree was described as a seedling. This case was investigated. Specimens of the disease were sent to Washington, but the trouble was not definitely identified as sun-blotch. This tree was apparently discarded.

The interesting and unusual history of the infected trees at Avon Park may indicate that certain avocado varieties might be hidden carriers of the sun-blotch disease. It was learned that the 30 trees in question were budded by the owner from scions of the Taft variety, obtained from a tree located in a grove near Lake Wales, Florida. This tree was apparently a highly productive strain of that variety and undoubtedly came ultimately from budwood or graftwood received from California, its place of origin.

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[3] Wolfe, H. S., L. R. Toy and A. L. Stahl. Avocado production in Florida. Fla. Agr. Exp. Stat. Bull. 272. 1934.

[4] Schroeder, C. A. Effects of sun-blotch on the anatomy of the avocado stem. Calif. Avocado Assoc. Yearbook 1935: 125–129. 1935.

Tip buds from the Taft tree in the Lake Wales grove were worked into West Indian seedlings in the Avon Park grove by the owner in 1924 or 1925. The resulting trees were later planted in the 2 adjacent rows in the grove. These trees, proving unsatisfactory, were afterwards topworked to Nabal and Taylor varieties, the buds or grafts being placed in the Taft trunks. The Nabal scions used in topworking the trees came directly from 2 reliable sources in California, the dealers stating that the budwood came from trees free from sun-blotch infection. All trees resulting from Nabal and Taylor scions placed in the Taft wood developed symptoms of sun-blotch in 1938 on fruit and foliage. The owner states that scions from this same lot of Nabal and Taylor budwood were placed in West Indian seedling and other stocks in this same grove and no sun-blotch has developed on these trees up to the present time.

The Taft stocks in these trees were suspected of being responsible for the development of sun-blotch in this case. It was learned, however, that no symptoms of the disease were recognized on the Taft foliage or fruits before the trees were cut back and reworked, and no recognizable symptoms were observed on a few remaining branches of Taft that were left on scattered trees.

The Taft tree at Lake Wales, whence the budwood was first obtained, was later visited and thoroughly examined for symptoms of sun-blotch. This is a large productive tree, planted 22 years ago. It has made a normal spreading growth and exhibits none of the dwarfed or drooping-branch symptoms characteristic of sun-blotch. No visible indications of the disease on leaves or fruit could be found. Other Taft trees in this same planting were examined but no symptoms could be detected. The large tree appeared perfectly normal and bore a fair crop of fruit. As the tree had not been sprayed this season, the fruit was severely affected with scab, and much of the foliage was attacked by Cercospora leaf spot.

This tree was one in a lot of 50 budded Taft trees obtained by the owner, in 1916, from a nursery in southern Florida. It is not known where the budwood for these trees came from, but, presumably, from California. The buds probably were placed in West Indian seedling rootstocks, as most of the local nurserymen made use of such stock at that time. Growth of trees in the grove was normal and healthy. Only two of the lot were satisfactory producers from the standpoint of crop production, *i.e.,* the large tree referred to and another nearly as large and adjacent to it. The original planting still remains, but most of the Taft trees show severe decline and have failed to produce fruit the past few years. Some have been severely pruned, but have failed to develop new vigorous growth. None has yet been topworked to other varieties.

SUMMARY

Definite cases of avocado sun-blotch have been observed in a single grove in Florida on Taylor and Nabal varieties topworked on Taft trees.

It is not possible at present to trace the infection back to any definite source. The Nabal scions were obtained directly from California, where the disease occurs on the Nabal in that State. It has not appeared in this grove on trees other than Taft, topworked simultaneously with the same budwood. Furthermore, other Taft trees reworked to Taylor developed typical symptoms of sun-blotch. The only infected trees found in the planting were those topworked on Taft wood. This indicates a possible relationship between the reworked Taft trees and the later appearance of the disease. No recognizable symptoms, however, were observed on a few remaining branches of Taft among the reworked trees.

It is generally known that an infected scion may transmit sun-blotch to the stock on which it is grafted; and this stock, if later rebudded or regrafted, may transmit the disease to the new top. Since no sun-blotch was observed on the Taft trees before topworking and no indications of the disease are evident at present on the original Taft tree from which budwood was taken, it is difficult to explain the appearance of the infection in this case unless it was carried in the Taft scions in a latent form.

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INFECTION OF TOMATO AND RED CLOVER WITH CONIDIA OF PLEOSPORA LYCOPERSICI AND MACROSPORIUM SARCINAEFORME[1]

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(Accepted for publication February 27, 1939)

INTRODUCTION

The tomato fruit rot, caused by *Pleospora lycopersici* Él. and Ém. Marchal, which affects California-grown tomatoes received in eastern markets, has been described by Ramsey (7). Although the disease causes considerable damage to eastern consignments of tomatoes, apparently no serious field losses are incurred on the green-wrap crop in California.

Tomato fruits are probably already infected at the time of packing and shipping, since vine-ripened tomatoes in fields from which green tomatoes were picked for shipment are found with typical symptoms of the disease. California tomatoes are inspected before being released, and no record of serious damage from this disease has been reported by shipping-point inspectors. Evidently the fungus is present in some of the fruits, but is latent, and does not become patent until after the fruit is packed for shipment to eastern markets.

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[1] Joint contribution from the Division of Plant Pathology, University of California, and the Department of Botany, University of Missouri.

[2] Grateful appreciation is expressed to Professor M. W. Gardner, Division of Plant Pathology, University of California, for advice and assistance.

Provided infection of tomato fruits take place prior to their shipment, the question of mode and source of infection arises. It was thought that fruit infection might originate from spores from saprophytic growth of the fungus on dead stems and leaves of the tomato plant; however, repeated isolations from such sources failed to yield the fungus. Although *P. lycopersici* has not been reported as a parasite of either tomato leaves or stems, it appeared advisable to undertake an investigation of its pathogenicity to that host.

INFECTION EXPERIMENTS

Young, healthy, greenhouse-grown tomato plants, *Lycopersicum esculentum* Mill., varieties Dwarf Champion, Earliana, San Jose Canner, and Stone, were atomized with a spore suspension of the conidia of *Pleospora lycopersici* in sterile distilled water. A parallel control series of plants were atomized with sterile distilled water. Both groups of plants were placed in a moist chamber. Five days later, small necrotic spots, usually 1 to 3 mm. in diameter, somewhat irregular in outline, and bordered by a diffuse yellow band, were observed on the leaves of the inoculated Earliana and Stone plants. Like symptoms were produced on San Jose Canner after 6 days, and after 8 days on Dwarf Champion. The fungus was reisolated and again proved pathogenic to tomato leaves upon reinoculation. Control plants remained healthy.

A similar experiment, with the same varieties and procedure as described above, was set up. Isolates of *Pleospora lycopersici*, obtained from diseased tomato fruits by G. B. Ramsey in Chicago were used. The results were alike in both experiments.

The imperfect stage of *Pleospora lycopersici,* as described by Marchal and Marchal (6), is *Macrosporium sarcinaeforme* Cav., a red-clover leaf-spotting organism. Isolates of *M. sarcinaeforme* from red clover, *Trifolium pratense* L., were obtained from Kentucky, Missouri, and Wisconsin. The pathogenicity of these isolates to tomato and various leguminous plants was tested.

Red clover, alsike (*Trifolium hybridum* L.), white clover (*T. repens* L.), alfalfa (*Medicago sativa* L.), and white sweet clover (*Melilotus alba* Desr.) plants were inoculated with a spore suspension of *Macrosporium sarcinaeforme* from red clover in one experiment and with conidia of *Pleospora lycopersici* in another. Suitable control plants were selected. All plants were placed in moist chambers. Eight days following inoculation, small, necrotic spots were observed on the leaves of all plants inoculated with *M. sarcinaeforme,* but no infection occurred on plants inoculated with conidia of *P. lycopersici.* The fungus was reisolated and proved pathogenic upon reinoculation. Controls remained healthy.

These results are in harmony with those of Bain and Essary (1) who infected red clover and alsike with *Macrosporium sarcinaeforme.* Elliott (3) was able to infect red clover, white clover, and alfalfa with the red-clover leaf-spotting fungus, but failed to infect onion (*Allium* sp.), muskmelon (*Cucumis melo* L.), and cowpea (*Vigna sinensis* (L.) Endl.). Krakover (6) succeeded in obtaining infection with *M. sarcinaeforme* on red clover only, though he inoculated alsike, crimson clover (*Trifolium incarnatum* L.), white clover, white sweet clover, alfalfa, pea (*Pisum sativum* L.), bean (*Phaseolus vulgaris* L.), hoary vetch (*Vicia villosa* Roth), potato (*Solanum tuberosum* L.), tomato, cucumber (*Cucumis sativus* L.), muskmelon, cabbage (*Brassica oleracea* L.), rape (*B. napus* L.), and lettuce (*Lactuca sativa* L.). Horsfall (4) secured infections of red clover, alsike, white clover, alfalfa, and sweet clover (*Melilotus* sp.) with the red-clover leaf-spot fungus.

Tomato and red-clover plants were atomized with a spore suspension of *Macrosporium sarcinaeforme* from red clover in one trial and with conidia of *Pleospora lycopersici* in another. Suitable controls were maintained. Typical spotting of red clover occurred after 8 days with *M. sarcinaeforme*

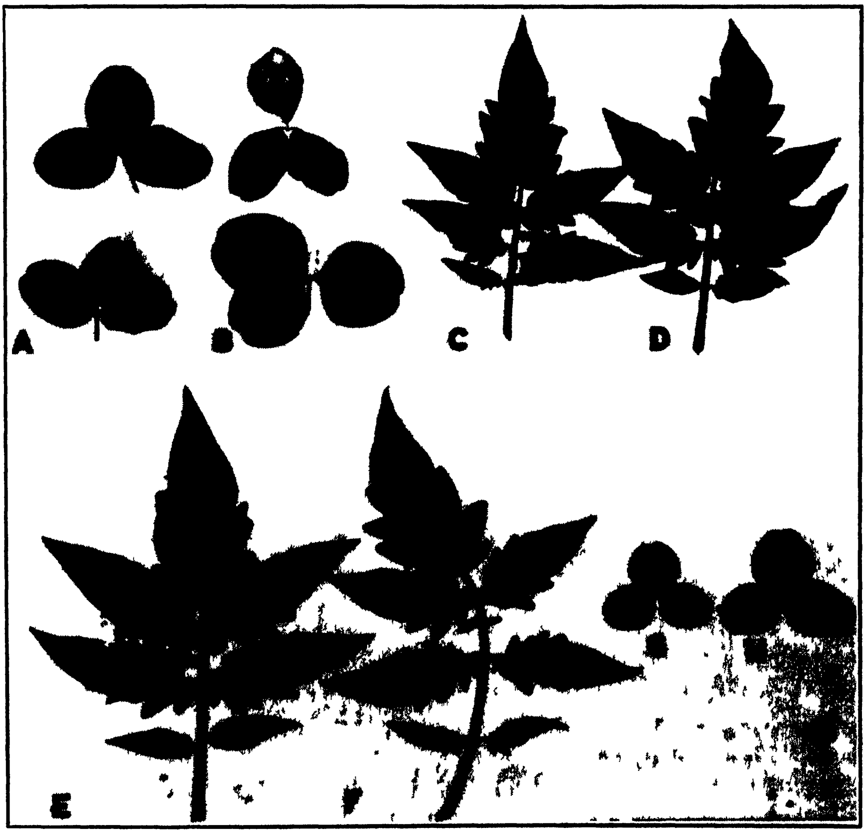

FIG. 1. A–D. Inoculation experiments with *Macrosporium sarcinaeforme* on red-clover and tomato leaves. A. Healthy red clover leaves. B. Typical spotting produced 8 days after inoculation. C. Healthy tomato leaf. D. Inoculated leaf; no infection 8 days following inoculation. E–H. Inoculation experiments with conidia of *Pleospora lycopersici* on tomato and red clover leaves. E. Healthy tomato leaf. F. Typical spotting produced 8 days following inoculation. G. Healthy red-clover leaf. H. Inoculated leaf; no infection 8 days following inoculation.

from red clover, but no infection of tomato occurred (Fig. 1, D). Typical spotting of tomato was observed 8 days following inoculation with conidia of *P. lycopersici*, but this fungus failed to infect red clover (Fig. 1, H). The fungus was reisolated in each instance and proved pathogenic upon reinoculation.

Tomato flowers were atomized with a spore suspension of *Macrosporium sarcinaeforme* from red clover. The results were negative. Other tomato flowers were atomized with a suspension of conidia of *Pleospora lycopersici*. The fungus proved pathogenic, causing considerable flower blight. A few flowers set fruits, which were picked when they attained 1 to 1½ in. in diameter. Small, black, necrotic spots occurred near the calyx end of such fruits. Isolations from the diseased tissue yielded pure cultures of *P. lycopersici*.

DISCUSSION

It is concluded that the imperfect stage of *Pleospora lycopersici* is incorrectly assumed to be *Macrosporium sarcinaeforme* by Él. and Ém. Marchal. Though, microscopically, the conidia and conidiophores of the tomato pathogen are not greatly unlike those of the red-clover organism, the differences in their cultural characters and pathogenic capacities are deemed adequate to disqualify the binomial *M. sarcinaeforme*, as applied to the imperfect stage of *P. lycopersici*. This is especially true in that *M. sarcinaeforme* was originally described by Cavara (2) as the cause of a leaf spot of red clover and has since been shown by several investigators to be pathogenic on certain leguminous plants only. *M. sarcinaeforme* from red clover produces an abundance of conidia in culture; aerial growth on agars is scant and usually closely appressed to the agar surface (Fig. 2, A). The perfect stage of this organism isolated from red clover has never been observed. *P. lycopersici* produces limited numbers of conidia in culture and copious and dense aerial growth, which is not appressed to the agar surface (Fig. 2, B). The perfect stage of the tomato pathogen is produced rather freely by all isolates observed by the writer.

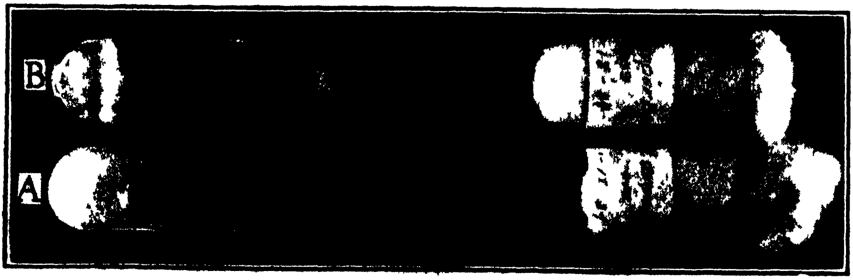

FIG. 2. Eight-day-old cultures of *Pleospora lycopersici* and *Macrosporium sarcinaeforme* grown at 25° C. on potato-dextrose agar. A. *M. sarcinaeforme*. B. *P. lycopersici*.

A possible mode of fruit infection has been demonstrated, namely, by conidial infection of tomato flowers, the fungus remaining latent in the pistil until the fruit has become fully developed. The source of inoculum for such infection may possibly be the spores present on the necrotic leaf spots produced on tomato leaves by conidial infection.

SUMMARY

Conidia of *P. lycopersici* are capable of infecting leaves and flowers of tomato upon inoculation; negative results were obtained when red clover, white clover, alsike, alfalfa, and white sweet clover were inoculated. Conidia of *M. sarcinaeforme* are capable of infecting various leguminous plants upon inoculation; no infection of tomato was obtained.

Differences in the cultural characters and the pathogenic capacities of the two fungi prohibit the application of the binomial *Macrosporium sarcinaeforme* to the imperfect stage of *Pleospora lycopersici*.

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OBSERVATIONS ON POWDERY MILDEW ON CULTIVATED BLUEBERRIES IN MASSACHUSETTS IN 1938

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The rapid expansion of commercial blueberry production in the last few years makes the matter of varietal differences, particularly with reference to their productivity under diverse environmental conditions, of greater importance. Among other factors affecting productivity are the ease and extent to which different varieties are attacked by powdery mildew (*Microsphaeria* sp.) under conditions that favor its development. Although it is difficult to evaluate the effect of infection by mildew on the productivity of a variety of blueberries, there seems little doubt that the more heavily affected plants are handicapped in their productivity as compared with noninfected ones. Blueberry growers and plant pathologists observing blueberries have known that different varieties vary greatly in their susceptibility to mildew, although no record of these differences is to be found. To partly fill in this lack, some observations have been made on the susceptibility of blueberry varieties to mildew in Massachusetts. In making these observations, 7 plantings well distributed throughout southeastern Massachusetts from Middleboro and Hanover to East Sandwich on Cape Cod were visited. Nearly all the blueberry plantings of bearing age in the State are now of small acreage, mostly about 1 to 1½ acres. Only 2 plantings exceed this area. Six or 7 varieties of blueberries are to be found in most of these plantings.

Much variation was observed in the amount of mildew in different plantings because of local environmental conditions. It is evident, however, that Pioneer is the most susceptible of the varieties now grown in Massachusetts. Cabot also is very susceptible but was noticeably less badly infected than Pioneer when growing with it. Wareham follows Cabot in susceptibility. These 3 varieties form a definite, well-graded series in which there is no question as to their sequence. In these three varieties mildew occurs mostly on the upper surface of the leaves. Concord, Jersey, and Rubel are intermediate in susceptibility. In several plantings very little mildew was found on Rubel and this variety would be rated as rather resistant, except that under some conditions it was found to be quite badly infected, showing that the variety is at least moderately susceptible to mildew. On the basis of present observations, Rubel appears to be more resistant than Wareham and more susceptible than Concord or Jersey. In both Rubel and Jersey, mildew appears mostly on the under surface of the leaves. Harding, Katherine, Rancocas, and Stanley appear to be very resistant to mildew, as these varieties were found to be nearly free from it, even in plantings with other varieties that were quite badly infected. Of the four varieties Harding and Katherine appear to be more resistant than the other two, and to be the most resistant of all varieties in Massachusetts. Further observations must be made, however, before a definite rating can be made for each.

The possibility of obtaining by selection from crosses varieties that are more resistant to mildew may be illustrated by a progeny test in the experimental blueberry planting at the State Bog. Among some 200 to 300 seedlings of a cross between Wareham and Pioneer a wide range of infection by mildew was observed in the late summer and fall of 1938, a season highly favorable to the development of mildew. On some of the plants most of the leaves were covered with mildew; other plants were almost entirely free from it, and between these extremes all gradations were to be found. Plants that had been selected as the best, because of size, flavor, appearance, and ease of picking of the berries, were also most nearly free from mildew. As one of the parents of this cross is a variety moderately susceptible to mildew and the other, the most susceptible of all varieties grown in Massachusetts, it is evident that varieties with much greater resistance to mildew may be obtained, even from crosses of such a parentage.

As a further example of the possibility of obtaining varieties more resistant to mildew by crossing, it is of interest to note that, although the two varieties Pioneer and Katherine are of the same parentage, Pioneer is the most susceptible and Katherine the most, or one of the most, resistant to mildew of all varieties grown in Massachusetts. The high degree of resistance in Stanley may be because of the fact that Katherine was one of the parents in the cross from which Stanley resulted.

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PHYTOPATHOLOGICAL NOTES

The Relation of Saperda tridentata to Infection of American Elm by Ceratostomella ulmi.—*Ceratostomella* (*Graphium*) *ulmi* (Schwarz) Buisman has been isolated from individuals of the insect species *Saperda tridentata* Oliv., the common elm borer.[1,2] Routine isolations made at this laboratory over a period of 4 years showed that many *S. tridentata* beetles, most of which emerged from diseased elm logs, carried the fungus. Some individuals carried large numbers of spores.

Saperda tridentata is very prevalent in the Dutch elm disease area adjacent to New York City. In this region the insects usually begin to emerge from decadent elms during the month of May. The adults feed largely on elm leafblades, midribs, petioles, and to some extent on newly forming, succulent shoots. Therefore, experiments were conducted in 1936, 1937, and 1938 to ascertain the effectiveness of Saperda feeding wounds as infection courts for *Ceratostomella ulmi.*

For this work 2- to 4-year-old budded elms (*Ulmus americana*) were obtained outside the known elm-disease zone. The trees were potted in 16- to 18-quart galvanized pails, either in the fall or spring preceding attempts at inoculation. The insects were obtained from decadent or diseased elm logs cut in the vicinity of Yonkers, New York.

Actively growing trees with mostly succulent and rapidly elongating shoots were brought to the greenhouse the last week of May. All wounds present (if any) were carefully covered with melted paraffin. Beetle feeding wounds were obtained as follows: two or 3 individual cages made of screen, or of celluloid material having open cloth ends, were attached to each tree so that one or more branches were included within each cage. The beetles were surface-disinfested and 5 to 10 placed in each cage. The cloth ends of the cages were then securely closed and the cages supported from above to avoid mechanical injury to the developing foliage. After 5 to 12 days the beetles and cages were removed.

Ceratostomella ulmi spores were introduced into Saperda feeding wounds by 2 methods: (1) In 1936 and 1937 experiments the wounded trees were atomized with a water suspension of spores immediately after removal of the beetles. (2) In the 1938 experiment some wounded trees were inoculated as in the first method; in addition to these, others were inoculated by *C. ulmi*-infested beetles during their feeding activities. For the latter method surface-disinfested beetles were immersed for 3 minutes in a water suspension of coremiospores before being placed in the above designated cages.

In each experiment the wounded, inoculated trees were divided into 2 lots; one lot was held constantly in the open greenhouse and the foliage

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[1] Collins, C. W. Insect vectors of the Dutch elm disease caused by the fungus *Ceratostomella ulmi* (Schwarz) Buisman. Proc. Nat. Shade Tree Conf. 11: 127–132. 1935.

[2] May, C. The Dutch elm disease from the research standpoint. Proc. Nat. Shade Tree Conf. 11: 122–127. 1935.

always kept dry, the other was subjected, immediately following inoculation, to certain moisture treatments. (See table 1 for descriptions of open-greenhouse, and moisture treatments.) After completion of the moisture treatments the second lot was placed with the first. Thereafter, both lots were held under the same open-greenhouse conditions, care being taken to avoid wetting any of the foliage.

All trees were cut in the fall of the year in which they were inoculated. The wood was then carefully exposed and examined in detail for fungous discoloration. Discolored tissue was excised and planted on nutrient agar in Petri dishes. Positive evidence of infection was based upon recovery of *Ceratostomella ulmi* from discolored wood.

Infection was obtained in 1 of 4 trees inoculated in 1936, 1 of 8 inoculated in 1937, and 10 of 20 inoculated in 1938 (Table 1). It is notable that, in 1938, 6 of 10 trees inoculated by means of beetles carrying *Ceratostomella ulmi* became infected. None of the infected trees wilted. With one exception discoloration was mostly scanty, consisting of small brownish to black spots in the wood of the youngest shoots, directly beneath wounds made by Saperda. In 1938 in one tree discoloration extended through most of the

TABLE 1.—*Inoculation of American elm with Ceratostomella ulmi spores through wounds made by Saperda tridentata*

Year	Beetles		Inoculation of wounds		Number of trees and treatment[b]	Number of trees infected	Date cut and tissue cultured
	No. per tree	Days of feeding	Method[a]	Date			
1936	26	12	Atomized	June 12	2—M.C. 2—O.G.	1 0	Oct. 2
1937	10	5	Atomized	June 8	4—Spr. 4—O.G.	1 0	Sept. 10 to 13
1938	10	5	Beetles	June 3 to 8	5—Ato.; Spr. 5—O.G.	2 4	Sept. 13 to 16
			Atomized	June 8	5—Spr. 5—O.G.	2 2	

[a] Inoculation method.
1. Atomized.—Wounds atomized with a water suspension of *C. ulmi* spores immediately after beetles removed from trees.
2. Beetles.—*C. ulmi*-infested Saperda beetles introduced spores through their feeding wounds.

[b] Treatment of trees after inoculation.
1. M.C.—Trees exposed for 11 days in a greenhouse moist chamber within which the relative humidity ranged from 85 to 98 per cent; then placed with and treated similar to O.G. lot.
2. O.G.—Greenhouse with open ventilators. Trees exposed constantly to open-greenhouse conditions and foliage was always dry.
3. Spr.—Trees sprinkled lightly with water, twice daily, for 7 days then placed with and treated similar to the O.G. lot.
4. Ato.; Spr.—Foliage on which beetles were feeding atomized with water daily. After beetles removed the trees were sprinkled lightly with water, twice daily, for 7 days then placed with and treated similar to O.G. lot.

wood of one wounded shoot. This tree was inoculated by atomizing Saperda wounds with *C. ulmi* spores after removal of the beetles. The failure of the fungus to invade the tissue extensively probably was because of the fact that the beetles fed upon the more succulent parts of growing shoots. Hypodermic injection of *C. ulmi* spores into such tissue on other trees has never resulted in extensive invasion.

Environment apparently exerted some effect upon the outcome of inoculation. In 1936 and 1937 the open-greenhouse temperature was abnormally high and relative humidity low. Only 1 tree became infected in each of these years, both trees having been subjected to the high-moisture treatments. In 1938, during the incubation period, the open-greenhouse temperature was usually 3 to 4 degrees lower and the relative humidity was higher than outside. Records kept during this period showed that in the open greenhouse the temperature ranged predominantly from 70° to 85° F., and the relative humidity from 60 to 90 per cent. These conditions appear favorable for infection because 6 of 10 trees inoculated in 1938 and held constantly under open-greenhouse conditions were infected.

From the results reported it is clear that wounds made by *Saperda tridentata* on growing elm shoots did serve as avenues of entrance for *Ceratostomella ulmi*. Inoculation by beetles carrying *C. ulmi* was slightly more successful than the wholly artificial method of atomizing the wounds with spores.—LEON J. TYLER, K. G. PARKER, AND L. L. PECHUMAN, Cornell University, Ithaca, New York.

Symptoms of Yellow Ring Spot and Longevity of the Virus in Tobacco Seed.[1]—It has been demonstrated that 2 strains of the tobacco ring-spot virus, yellow and the ordinary green,[2] are transmitted through the seed of tobacco. These viruses belong to the same virus group as evidenced by the similarity of symptoms, by serological tests, and by the fact that when one strain of the virus becomes systemic in a plant it appears to be protected against invasion by other strains, except in uninvaded tissue.

The virus appears to be quickly inactivated by drying or by standing for relatively short periods *in vitro;* but the fact that it is transmitted through seeds suggests that it, in common with some other seed-transmitted viruses, will withstand relatively long aging when the virus is associated with the living plant cells. In the present study it was desired to determine whether the ring-spot virus retained activity in seeds of tobacco after storage for several years.

About 2 weeks after sowing seed of a yellow ring-spot plant, the cotyledons of a few seedlings turn yellow, if the seed is relatively fresh, or the first true leaf may be the first part to bleach if the seed is older. As the seedlings develop they become distinctly yellowish and may be recognized by this character throughout the life of the plant. Rings or line patterns rarely develop on these plants, but transfers from them to healthy plants cause the typical ring pattern. Seed transmission of the yellowing strains of the virus may, therefore, be readily studied in spite of the fact that ring patterns rarely develop in infected plants. The disease of tobacco caused by the so-called ring-spot virus can go from one plant generation to another indefinitely, without the production of any of the ring patterns commonly thought to be the chief symptom of the disease. It seems obvious, therefore, that the ring-pattern symptom is an inoculative and invasive symptom and that the true symptoms are manifest only after the virus has entered the growing-point leaves and become truly systemic. The yellowing strain then causes partial bleaching of the leaves with sometimes leaf-edge necrosis, and the green strains merely the leaf-edge symptoms, under certain conditions not well understood, and a slight stunting and darkening of the plant.

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[2] By ordinary green strains is meant the common field ring spot similar to that studied by Fromme, Wingard, and Priode (Fromme, F. D., S. A. Wingard and C. N. Priode. Ringspot of tobacco; an infectious disease of unknown cause. Phytopath. 17: 321–328. 1927). It is not considered to be a distinct strain as Smith assumes it to be (Smith, K. M. A textbook of plant virus diseases. 615 pp. P. Blakiston's Son & Co., Philadelphia, 1937).

Both the yellowing and the ordinary green strains of the virus following so-called recovery have a marked effect on pollen, causing varying degrees of abortion, which is reflected in a reduced set of seed. Some strains cause complete pollen sterility and affected plants fail to set any seed whatever, unless cross pollinated.[3]

It is interesting to reflect on the interpretation that might have been placed on attempts to reinoculate yellow or green ring-spot plants with any strain of the ring-spot virus, if the disease originally had been discovered as a seed-transmitted one, and the bleaching, leaf-edge, and sterility symptoms rather than the ring-spot symptoms had been stressed as the typical symptoms of the disease. The hypothesis of recovery and the development of immunity would hardly have been advanced.

Seed transmission of the ordinary green strains is not easily recognized

TABLE 1.—*Reduction of seed transmission of yellow ring spot in a lot of seed with aging*

Plant	Days after harvest	Green plants	Yellow plants	Percentage seed transmission
Turkish	0	182	25	12.1
"	27	829	80	8.8
"	58	753	60	7.0
"	1048	1005	33	3.2
"	1841	386	0	0.0

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[3] From time to time attempts have been made to produce first-generation hybrid tobacco seed in sufficient quantity for commercial crops, on the theory that the F_1 plants are more desirable than either parent. This entails emasculation and hand pollination of all blooms. This process could be greatly facilitated if all seed plants were inoculated when young with a yellowing strain of the ring-spot virus that caused complete pollen sterility. Then only pollination and not emasculation would be required. If any seed transmission occurred the affected plants would be completely eliminated, as they grow very slowly in comparison with healthy plants.

because plants affected through the seed usually do not develop ring patterns and affected plants usually retain their normal green color for the most part. They may sometimes be recognized by the development of chlorotic or necrotic leaf-edge symptoms on the tip half of the leaves. Therefore, studies on the longevity of the ring-spot virus in seeds have largely been confined to the yellowing strain.

In table 1 are given the results obtained with one lot of seed of Turkish tobacco. These results suggest that the virus gradually becomes inactivated with time but do not prove it, for it is possible that infected embryos lose their power of germination more rapidly than noninfected ones.

TABLE 2.—*Seed transmission of yellow ring spot in young and old seeds*

Plant	Days after harvest	Green plants	Yellow plants	Percentage seed transmission
8278 B	262	220	4	1.8
"	1569	1280	4	0.3
7795 sdlgs. 3 pl.	38	316	40	11.2
"	1892	1057	15	1.4
Sdlg. of R 45 Dark.	38	207	8	3.7
"	1904	1267	25	1.9
7795 Q	60	1235	12	1.0
"	1954	900	4	0.4
7795 pl. 2	195	204	1	0.5
	2012	31	1	3.1
7795 Q	173	1248	10	0.8
"	2064	1284	1	0.1

In table 2 are given the results obtained with 6 lots of seed during the first year and after several years of storage. These results prove that the yellow ring-spot virus is not inactivated in seeds after a period of 5½ years. It will be of interest to determine whether or not the virus has become inactivated when the seeds have lost their power of germination.—W. D. VALLEAU, Kentucky Agricultural Experiment Station, Lexington, Ky.

Observations on Fructification of Ceratostomella ulmi in England.[1]—During the past 3 years the writer observed the fructifications of *Ceratostomella ulmi* (Schwarz) Buisman under natural conditions in England. Dead, dying, and fallen elms and woodpiles containing elm were numerous. Coremia and perithecia of *C. ulmi* were found in great numbers in all parts of the galleries of *Scolytus scolytus* Fab. and *Sc. multistriatus* Marsh., on the inner surface of the bark, between flakes of inner bark, on the outer xylem surface, and on cut and broken surfaces of infected elm wood. The mycelial-conidial stage of *C. ulmi* was found also between the loosening bark and the outer xylem surface on a few occasions.

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[1] Laboratory accommodations for this study were furnished by the Imperial Forestry Institute and the Hope Department of Entomology, Oxford University, and this hospitality is gratefully acknowledged. The writer is indebted to D. E. Parker, Associate Entomologist, Division of Forest Insects, Bureau of Entomology and Plant Quarantine, for counsel and assistance in the field work and critical reading of the manuscript.

The purest, densest, and most extensive stands of coremia and perithecia were found on the outer surface of elm wood from which the bark had just begun to loosen. This stage of decadence of the elm is of prime importance to the abundant fructification of *C. ulmi,* and heavy attack by bark beetles obviously hastens the necessary degeneration. In most cases elm wood that became severely affected by *C. ulmi* and infested by bark beetles during a given year produced its most extensive stands of coremia and perithecia during March and April of the next year. Trees that were only mildly affected by *C. ulmi* or were apparently unaffected when felled during late September did not yield extensive fructifications until the next August and September, while similar trees, felled in August or early September, sufficiently early to become well infested by bark beetles before the end of their seasonal activity, yielded abundant coremia and perithecia the following spring.

It appeared that the moisture content of the substratum must be relatively high to favor abundant formation of coremia and must remain high for several days to favor perithecial formation. Elm wood in contact with moist soil showed more extensive fructification of the fungus than did standing dead trees. During summer months extensive fresh stands of coremia were found on standing dead elms only after heavy or protracted rains.

Young coremia could be found at any time of the year, but, obviously, their development ceased temporarily during periods of freezing temperatures. Three years' field observations, however, indicated that the development of perithecia ceased during November and did not begin again before about the middle of March. The earliest dates on which perithecia were found with ascospore slime exuding were April 15, 1936, April 9, 1937, and March 31, 1938.

Most of these observations on the relation of environmental factors to fructification of *Ceratostomella ulmi* were made in the Oxford district, where March does not differ greatly from February in mean temperature and rainfall but does have an average of 111 hours' bright sunshine as compared with 67 during February. During the 61 days of March and April the mean temperature in the New York City area is approximately the same as that of the Oxford district, but New York has about twice as much rain and 200 hours' more sunshine.

Perithecia commonly developed in greatest numbers on surfaces that had produced dense stands of coremia 2 to 4 weeks previously. Therefore, it is considered that the perithecia are undoubtedly important in extending the time during which a given bark or wood surface is well supplied with fresh spores of *Ceratostomella ulmi.* A few collections of bark and wood having pure stands of perithecia but showing no signs whatever of a previous stand of coremia were sufficient to indicate, however, that perithecia need not be preceded by coremia on the same surface. It was commonly noted that wherever perithecia started to develop following a stand of coremia, the area was not overrun by the common saprophytic fungi of such habitats until the perithecia had matured. But stands of mature coremia and perithecia were nearly always broken down within a few days by the mites, sowbugs, dipterous larvae and other crawling inhabitants of decadent elm under the natural conditions of England.

Coremia of *Ceratostomella ulmi* were found once in nature on a substratum that could properly be called outer bark. These occurred on a completely shaded and very moist fallen branch from which birds had pecked larvae of bark beetles. Because 2 mm. was the greatest distance from the edge of the nearest bird-pecked hole at which a coremium was found on this material, it appeared likely that *C. ulmi* had advanced from inner bark to the outer surface by mycelial growth through the small holes. Perithecia were never seen on the outer bark surface of elm under natural conditions, but they developed on the outer bark of moist-chambered ¼″ branches of *Ulmus americana* that were killed by 1937 infection and collected in late May 1938. —JAMES M. WALTER, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

Dissemination of Celery Blight Pathogens on the Clothing of Farm Laborers.—The rôle of certain insects and animals, including man, in the dissemination of specific plant pathogens has been reported from time to time by several workers. Martin,[1] in particular, has shown that tomato pickers may carry many spores of the tomato leaf-spot fungus, *Septoria lycopersici* Speg., on their hands and garments. He considers them important factors in the dissemination of this fungus.

The writer obtained evidence in 1937 showing that the spores of the early and late blight pathogens of celery, *Cercospora apii* Fresen. and *Septoria apii* Rostr., are carried similarly on the clothing of farm laborers.

On Staten Island, New York, it is a common farm practice to intercrop celery and spinach, *i.e.*, alternate 1 row of celery with 2 rows of spinach throughout a given bed. When the spinach is cut, the celery is usually from one-half to two-thirds mature. During August two such beds, the celery plants in which were affected by early and late blights, were observed. The celery in one bed from which the spinach had been cut about 10 days previously was severely blighted, while the celery in the other bed from which the spinach had not yet been cut was considerably less blighted. The celery plants in both beds were from the same seed bed and had received the same number of applications of 20–80 copper-lime dust. The grower concerned made a practice of cutting the spinach in the morning when the plants were wet with dew. This suggested the theory that the spinach cutters had disseminated the spores by means of their clothing.

This theory was given further support by the observations of a prominent celery grower, Mr. A, near Sodus, N. Y. This grower states, that, on a morning when there was a heavy dew, his neighbor, Mr. B, after walking through his own severely blighted celery, walked diagonally across part of A's celery field. In 2 or 3 weeks blight appeared in a diagonal strip across A's field corresponding to the path taken by B. The rest of the celery, according to Mr. A., had developed no blight symptoms.

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[1] Martin, W. H. Dissemination of *Septoria lycopersici* Speg. by insects and pickers. Phytopath. 8: 365–372. 1918.

In order to find out if both early and late blight spores could be picked up and carried readily on cloth, a piece of muslin (3 sq. ft.) was dragged over the tops and sides of several severely infected plants and then drawn over the tops of 48 young blight-free celery plants, approximately 6 inches tall, previously syringed with water. As a check a similar but noncontaminated cloth was drawn over the tops of a second series of 24 young plants syringed as before. The first cloth was then rinsed in water and examined for spores. Spores of *Cercospora* and *Septoria* were found in abundance. The inoculated and check plants were maintained in an atmosphere of approximately 100 per cent relative humidity by covering them with orange boxes wrapped in heavy burlap, which was kept soaked with water for a period of 48 hours. The boxes were then removed. Two weeks later all inoculated plants showed definite symptoms of both blights, while the checks remained healthy.

Further corroboration was furnished by the results of another experiment in which the inoculating medium was the wet coat sleeve of a farm laborer who had been cutting spinach from between rows of celery affected with early and late blights. The coat sleeve was dragged over the tops of 24 young blight-free plants that, together with a like number of noninoculated check plants, were treated as previously related. Both diseases had developed on the inoculated plants at the end of 2 weeks, while the checks remained healthy.

The results of these experiments prove rather conclusively that considerable risk attaches to walking through or cultivating celery fields when the plants are wet, whether from dew, rain, or irrigation water. Failure of some growers to control one or both blights, despite frequent applications of sprays or dusts, may perhaps be explained on the basis that they have at some time unintentionally disseminated the blight pathogens throughout their fields in this manner.—M. B. LINN, *Department of Plant Pathology, Cornell University, Ithaca, N. Y.*

Studies of Monosporous Cultures of Septoria bromigena.[1]—Among 200 monosporous lines of *Septoria bromigena* Sacc., isolated from *Bromus inermis* Leyss. in the spring of 1937 and subsequently grown on twelve artificial media, there were many consistently different cultural types that fell into 4 groups, corresponding with the locality whence the lines were isolated.

Isolations were made from material collected at 4 localities in Minnesota: University Farm, St. Paul; Coon Creek; Dundas; and Waseca. From each collection 50 single spores were isolated by means of a micromanipulator. Each spore was placed on a small smear of potato-dextrose agar on the lower surface of a cover slip on a van Tieghem cell. When the spore had germinated and produced a small patch of mycelium, usually

[1] Paper No. 1689 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station. Assistance in the preparation of these materials was furnished by the personnel of Works Progress Administration Official Project No. 465–71–3–350.

within 2 days, a transfer was made to 125-cc. Erlenmeyer flasks containing 30 cc. of potato-dextrose agar.

The isolates grew very slowly on artificial media, requiring 60 days to cover the surface of a flask containing 30 cc. of medium. Twelve artificial

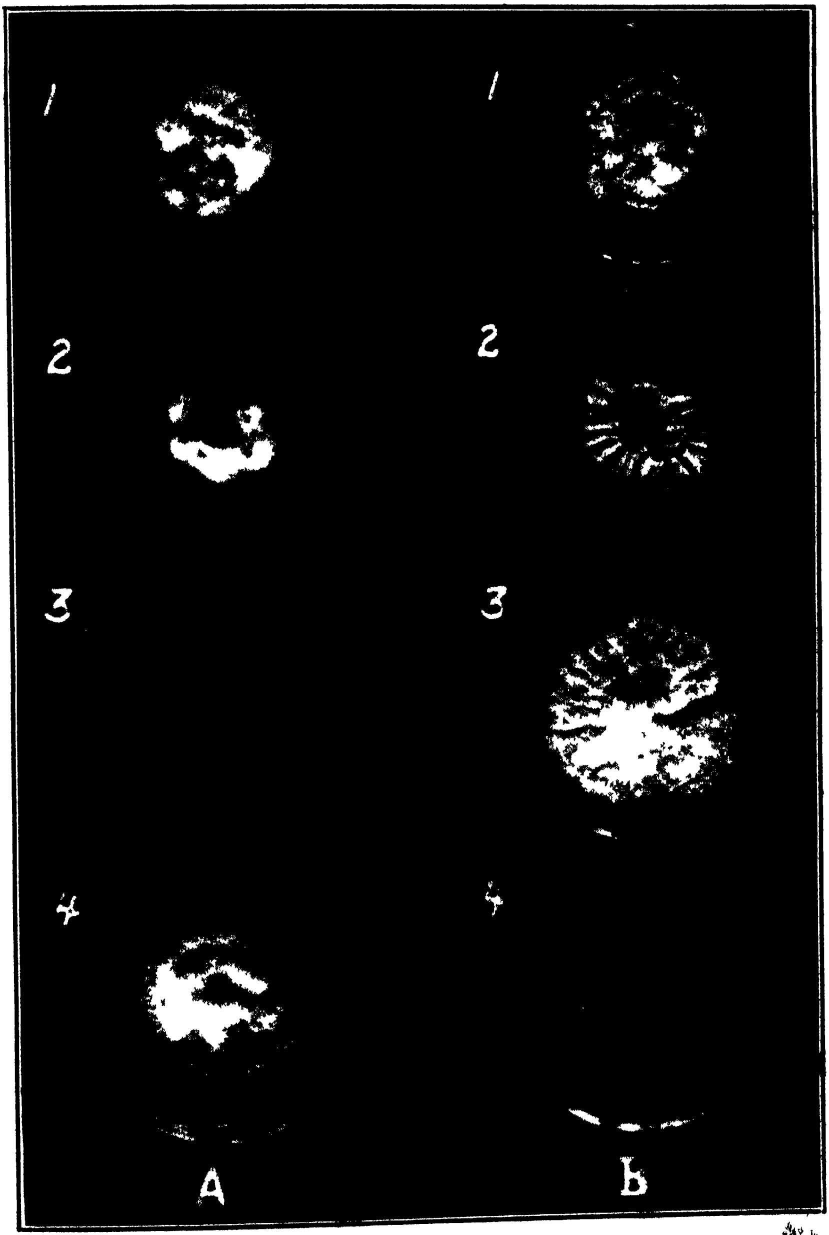

FIG. 1. A. Monosporous isolates from (1) Waseca, (2) Dundas, (3) Coon Creek, (4) University Farm, Minn. B. 1, 2, 3, and 4: strictly mycelial variants from different monosporous cultures.

media were tested, and potato-dextrose agar (390 gm. potatoes, 15 gm. agar, 20 gm. dextrose, 1 liter of water) was found to be as suitable for growth as any, so it was used throughout this study.

The isolates from each locality differed from those from every other locality, while those from the same locality were very much alike, though differing slightly (Fig. 1, A). The isolates from University Farm produced short, pink aerial hyphae, and had an outer zone of dark, leathery mycelium. The Coon Creek isolates produced a tough, leathery mycelial mat with very short, dark-grey to brick-red aerial hyphae. The Dundas isolates produced dark aerial hyphae over a rough mycelial mat. The Waseca isolates produced white and dark aerial hyphae over a solid leathery mycelial mat. In all isolates pycnidia were produced abundantly. These characters have remained constant for 18 months, through successive transfer on potato-dextrose agar.

Sector variants appeared in some cultures of all monosporous lines, several distinct variants often developing in a single culture. These variants were either entirely mycelial or they produced pycnidia sparingly. Color of variants grown on potato-dextrose agar ranged from delicate shades of pink to general hues of white and purple. The strictly mycelial variants (Fig. 1, B) have remained constant through successive transfers for 18 months; changes in temperature, light, hydrogen-ion concentration, and nutrients have failed to induce the formation of pycnidia. Variants producing pycnidia often reverted to their parental type, or, in some instances, gave rise to other sector variants. The pigments formed in all cultures were diffused into the medium and were water soluble.

In the greenhouse, plants of *Bromus inermis,* known to be susceptible to *Septoria bromigena,* were inoculated by macerating the mycelial mats of 6 nonsporulating variants in sterile distilled water and injecting this mycelial suspension hypodermically into the growing spindles. No infection resulted. Check plants inoculated with suspensions of conidia from parent cultures became severely infected.

Studies on the pathogenicity of the isolates from different localities are underway. Preliminary evidence has indicated that *B. inermis* is the only susceptible species of 34 species of *Bromus* tested and that inbred lines of this species differ greatly in susceptibility.

A more detailed account of these investigations will be published later. LEWIS ALLISON, University Farm, St. Paul, Minnesota.

Ceratostomella ips Associated with Ips lecontei in Arizona.—Observations made in the fall of 1938 showed a blue-staining fungus commonly associated with *Ips lecontei* Sw. attacking ponderosa pine, *Pinus ponderosa* Dougl., in the Prescott National Forest, Arizona. The injury was rather widespread in this forest where numerous trunk-infested trees, 4 to 12 inches in diameter at breast height (4½ feet) were being killed.

Examination of dead or dying trees always showed the sapwood markedly stained. In trees still apparently healthy but recently attacked by the bark beetles, the stain was found to originate at the insect tunnels. The sapwood adjacent to the galleries was often abnormally infiltrated with resin, a condition which seemed to precede the appearance of the stain.

The blue-staining fungus *Ceratostomella ips* Rumbold was isolated from adult *I. lecontei* beetles and from the wood. Collections for culturing were made from 10 scattered trees in 2 of the most heavily infested areas on the forest. The living adult beetles were taken from standing trees, still green and, for the most part, from fresh galleries that yet showed no evidence of the stain. They were washed for a few seconds in 50 per cent alcohol and dropped onto malt agar slants in the field. *Ceratostomella ips* was isolated from 45 of 54 beetles cultured. Small pieces of both stained and unstained wood taken from the edges of fresh galleries consistently yielded the same fungus in culture. Yeasts were also secured from most of the insects.

The occurrence of *Ceratosomella ips* on ponderosa pine and its association with several species of *Ips* has been reported.[1] So far as can be determined, however, its association with *Ips lecontei* and its occurrence in the Southwest have not been previously recorded. The identification of the insect was confirmed by Dr. M. W. Blackman of the Bureau of Entomology and Plant Quarantine and that of the fungus by Dr. Caroline T. Rumbold of the Division of Forest Pathology, Bureau of Plant Industry.—DON E. ELLIS, Civilian Conservation Corps with the Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Albuquerque, New Mexico.

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[1] Rumbold, Caroline T. Three blue-staining fungi, including two new species, associated with bark beetles. Jour. Agr. Res. [U. S.] 52: 419–437. 1936.

BOOK REVIEW

Appel, O. *Handbuch der Pflanzenkrankheiten, Pflanzenschutz.* Vol. 6, part 2, pages 289–576. Paul Parey's Verlag, Berlin, Germany. 1938. Price, paper cover, 16.60 Rmk. (25 per cent reduction in U. S. A.)

The second part of volume six of the Handbook for Plant Diseases continues with the discussion of plant protection under avoidance and control measures. The first 44 pages concludes the material on physical control measures used to ward off pests. Mentioned are the use of barriers such as screens, fish nets, sand, dust, lime, and similar materials. The use of scare-crows and various materials, such as strips of paper, tin sheets, and mirrors strung on strings and run through fields to frighten birds and insects are described. Farm-management methods and measures used for protection from weather or climatic conditions are discussed. The use of apparatus and means for collecting and trapping pests based on protective migratory instincts, light attraction, nesting and food instincts are then taken up. The discourse is concluded with a summary of the utilization of dry and moist heat, cold, electricity, magnetism, and radiation.

The main part of the number is devoted to a broad review of chemical control measures. The discussion is divided into the following 3 parts: Chemical plant-control materials; biological testing of plant- and storage-protective materials; and results of the physical and chemical tests.

The portion devoted to chemical plant-control materials is divided into 3 parts, *i.e.*, inorganic elements, organic elements, and inert or carrier materials. Under inorganic elements is given a detailed discussion and summary of the following: 1, copper, mercury, lead, and thallium compounds; 2, potassium, sodium, ammonium, calcium, barium, magnesium, aluminum, zinc, manganese, iron, chromium, and cerium compounds; 3, arsenical compounds; 4, sulphur and sulphides, including carbon bisulphide; 5, phosphorus and phosphides; 6, fluorene compounds; and 7, chlorates, chlorides, and hypochlorides. A fuller account of the history, uses, poisoning effects, and chemistry is given for the copper, arsenical, and fluorene compounds. The second phase on organic materials includes a review of the following: gas-forming organic compounds, organic chlorines and chlor-nitro compounds, phenol, chlor- and nitrophenoles, hydrocarbons, mineral and tar oils, and organic bases and alkaloids. Following this is a full account of nicotine and nicotine materials. Herein is discussed the importance, the chemistry, the source and preparation, the physical and chemical uses, and the poisoning effects of the various materials. Then a compilation is given of the following nitrogen-free drugs: Derris, cube, Pyrethrum, Quassia and Scilla. Finally, the alcohols, ketones, acids, esters, organic sulphur compounds, furines, and dyes are reviewed. The third part of the presentation of the chemical plant control materials includes a discussion of the inert or carrier materials added to fungicides and insecticides. A review of stickers, spreaders, emulsions, protective colloids, carbonic and sulfonic acid salts, proteid materials, carbohydrates, glucosides and various other organic and inorganic materials is presented. Soluble and insoluble fillers or carriers and a review of materials, such as color, odor and flavor substances, added to attract insects and to protect man are then discussed.

The second main division of the book deals with the biological testing of plant and storage protective materials. Under this phase is taken up the testing of fungicides, weed killers, insecticides, and materials used for the treatment of pests and storage products. Methods for testing nematode and mice control products are included.

The third and last division of the book summarizes the results of the physical and chemical tests. The properties and characteristics of dusting materials, spray materials and a few other special materials are given.

Part 2, volume 6 of Dr. Appel's newly revised Handbook of Plant Diseases presents a complete review of chemical control measures. A detailed and up-to-date picture is given of materials and methods. The volume includes the basic world literature dealing with the subject and should be extremely useful to all students of plant protection.—**Otto A. Reinking**, N. Y. State Agr. Exp. Stat., Geneva, New York.

WEB-BLIGHT, A DISEASE OF BEANS CAUSED BY CORTICIUM MICROSCLEROTIA

GEORGE F. WEBER

(Accepted for publication March 4, 1939)

INTRODUCTION

The first authentic report of web-blight in Florida, and probably in the United States, was that of July 10, 1932, on Fordhook Lima beans growing at LaCrosse (14). Here the disease was found affecting several adjoining bean fields aggregating about 12 acres. It was destructive in these fields at that time. Each year since it has caused economic losses to growers in that and other distant vicinities. The investigations here reported were conducted to gain definite information concerning this new, increasingly destructive, disease.

THE DISEASE

Geographical Distribution.—Web-blight of beans has been found on different farms at LaCrosse, Alachua County, Florida, where it was distributed over a more or less circular area approximately a mile in diameter. It also has been found on several farms in a like area near Florahome, Putnam County, in scattered fields over an area several miles wide and about 10 miles in length in Seminole County, and near Belle Glade, Palm Beach County. A survey of all the bean-growing sections has not been made. It has been found on beans in Puerto Rico, (7), (8), and on other hosts in Texas, Louisiana, Alabama, Florida, India, Burma, Ceylon, Japan, and Philippines (1), (9).

Economic Importance.—The disease was generally distributed in Lima bean plantings at LaCrosse in the early summer of 1935, but caused no material damage until after the first picking, the last week in June. Seasonal rains during the next week so favored its spread as to cause an estimated loss of 66 per cent. The field was abandoned. A similar loss was caused by the disease in that section during each of the next two years.

At Florahome, in 1935, a 15-acre field of snapbeans, which, based on yields from noninfected fields, would have yielded more than 200 hampers per acre, was a total loss. The disease killed the plants before the most mature pods were large enough to harvest (Fig. 1). In the following year a 60-acre field in the same vicinity planted successively in 10-acre blocks each week showed more than 50 per cent loss from this disease. The following table shows data obtained on a survey trip in the Sanford section during 1936.

One grower picked 400 hampers of beans during the morning and did not remove them from the field until the next day. They were thoroughly wet by a light rain during the afternoon, and nesting developed to such an extent during the night that they were rejected at the packing house. These

[VOLUME 29, NUMBER 7, JULY, 1939]

FIG. 1. Portion of a bean field showing complete defoliation by web-blight.

heavy losses occurred during periods of high temperature and high relative humidity.

TABLE 1.—*Acreage, per cent of infection, and loss caused by web-blight of beans in parts of Seminole County, Florida, in October, 1936*

Owner	Location	Acreage	Percentage infection	Percentage loss
Gantz	Monroe	4	50	10
Bell	Monroe	6	20	4
Giles	Monroe	3	100	90
Cain	Monroe	2	15	1
David	Sanford	2	100	90
David	Sanford	2	5	0
Steele	Sanford	4	100	85

Host Range.—Web-blight was first observed in a 6-acre commercial field of Fordhook Lima beans, and later it was found on snap beans of the Bountiful, Giant Stringless, and Black Valentine varieties. It has been observed as a parasite, killing all or part of the above-ground portions or the foliage and fruit of the following plants:

Amaranthus retroflexus L.
Ambrosia elatior L.
Boehmeria drummondiana Wedd.
Cirsium nuttallii (DC.) A. Gray
Doidella teres (Walt.) Small
Echinochloa colona (L.) Link.
Eleusine indica (L.) Gaertn.
Emelista tora (L.) B. & R.
Ficus carica L.
Firmiana platanifolia (L.) R. Br.
Glycine apios L.
Melothria crassifolia Sm.
Persicaria portoricensis (Bert.) Sm.
Phaseolus lunatus var. *macrocarpa* L.

Phaseolus vulgaris L.
Pityothamus angustifolius (Gray) Sm.
Rubus cuneifolius Pursh.
Sambucus simpsonii Rehd.
Saururus cernuus L.
Syntherisma villosum Walt.
Vernonia gigantea (Walt.) Trek.
Vigna sinensis (L.) Endl.
Xanthium americanum Walt.

Some of these hosts became infected through contact with infected bean plants. Others were found growing in bean fields, along road sides or fence rows, and in the woods, far removed from cultivated fields.

Positive results obtained from inoculation experiments have shown that the fungus will parasitize a wide range of crop plants if inoculated when growing rapidly under optimum infection conditions of temperature and humidity.

Symptoms.—Web-blight of beans is characterized by the presence of many small, brown sclerotia and spiderweb-like mycelium on the stems, pods, and foliage. The primary infection of bean leaves is very similar to that reported by Matz (7, 8), on bean and (4, 6), on fig; and is indicated by the appearance of small, circular, watersoaked spots on any portion of the blade. Invaded cells die by the time the spot is 1 mm. in diameter. Such spots are much lighter in color than the surrounding unaffected tissue and appear to have been scalded. These areas later become tan-brown surrounded by a darker border, enlarge up to 3 cm. in diameter, and frequently appear somewhat zonate. In most instances there are an apparently superficial speck—a sclerotium—in the center of each spot, sand grains entwined by mycelia, or merely a roughened, ruptured area of the epidermis of the leaf blade. As the infected area becomes larger, light tan-color hyphae develop on the 2 surfaces of the spot, being more numerous on the side of the leaf that was continuously in a more humid location. These hyphae grow rapidly over the surface of the noninfected area of the blade, gradually killing it. Under favorable conditions the hyphae spread from leaf to leaf, stem, petiole, peduncle, flower, and fruit, tying them together with a web or mat of tenacious mycelial strands (Fig. 6, A). Web-blight, which name typifies its appearance, is suggested as a common name in contrast to thread-blight, the common name of the disease of woody perennials caused by *Corticium stevensii* Burt. The growth is mostly inconspicuous in dry weather except on the dead leaves. During humid periods the whitish hyphae spread out fan-shape from the point of infection and produce the conspicuous and destructive phase of the disease (Fig. 2). In this way the disease spreads from the points of primary infections in bean fields. In a few days, under favorable conditions, a field becomes a total loss if a large percentage of the plants carried primary infections at the same time.

The mycelial growth occurring on the petioles and stems often is a slight yellow-brown in the parts involved. Later these areas become tan or brown, slightly sunken and enlarge rapidly until they attain a length of about 2 cm., when they usually have girdled the stem, petiole, or peduncle. At this stage the margins of the spots are dark and their surfaces are covered

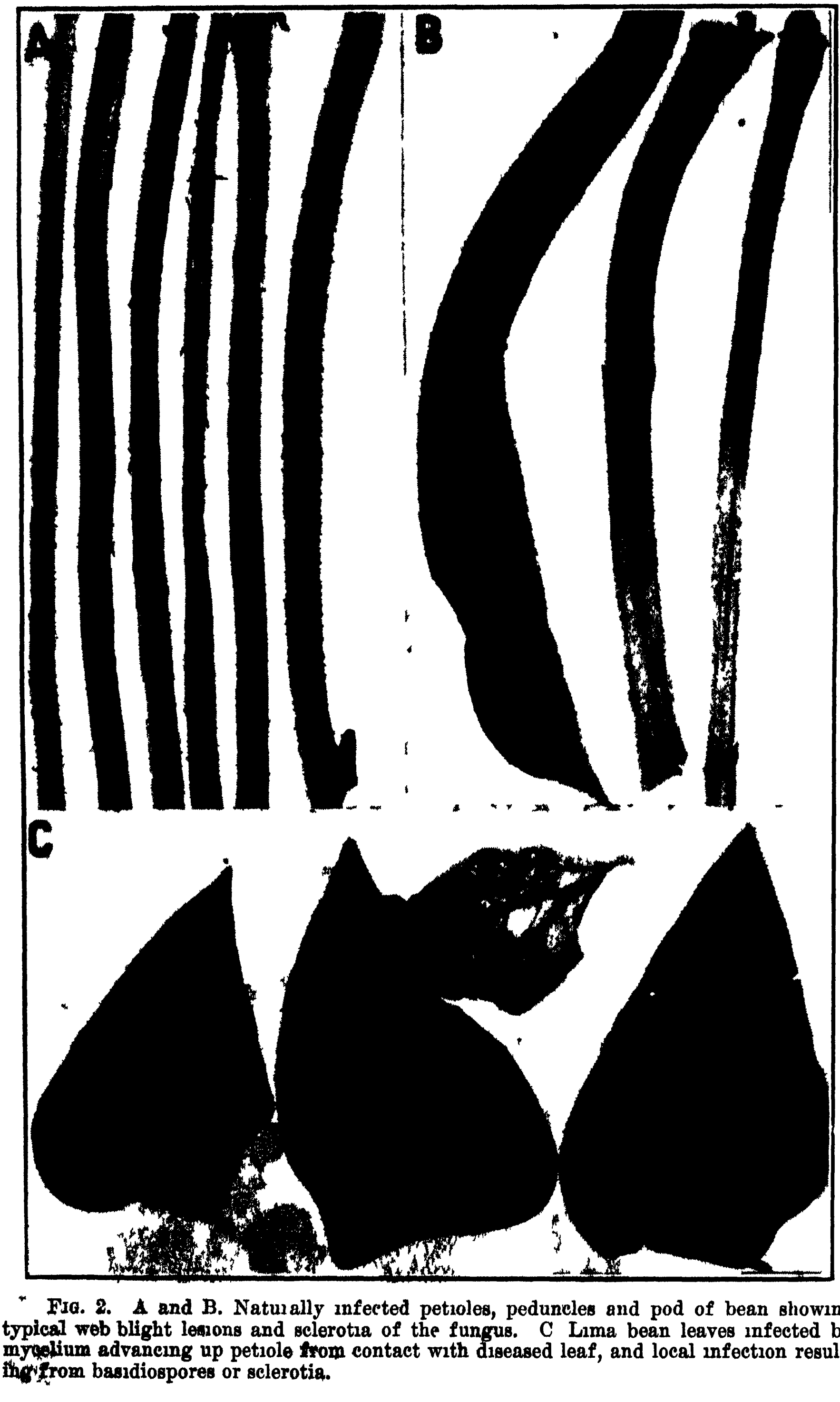

FIG. 2. A and B. Naturally infected petioles, peduncles and pod of bean showing typical web blight lesions and sclerotia of the fungus. C Lima bean leaves infected by mycelium advancing up petiole from contact with diseased leaf, and local infection resulting from basidiospores or sclerotia.

with a fine, close-growing, entangled hyphal web that bears the brown, light sclerotia. The pods are attacked in all stages of their development. The early infections on the young pods appear as light tan, irregular-shape specks, which often enlarge and coalesce, killing the pod. On more mature pods the spots are dark brown, more or less circular, slightly zonate and definitely sunken with a darker border and in this stage appear almost identical with anthracnose (Fig. 3). When numerous, they involve the entire pod; when scattered, the pod spots expand up to one centimeter in diameter, or as wide as the pod, and 2 or more centimeters in length producing a more or less oval-shape lesion. The older pods continue to develop after being attacked, unless the infection be located close to the stem end or had penetrated deeply into the pod tissue. The ovules within the pod that hap-

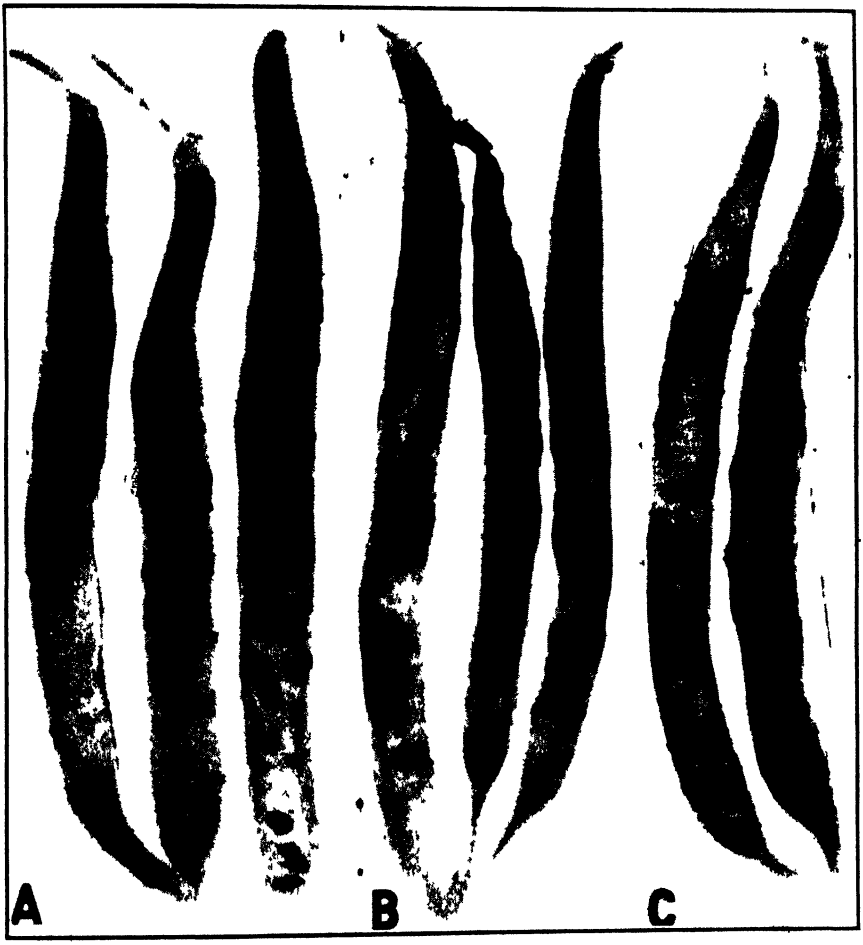

FIG. 3. Disease of pods of giant stringless bean caused by *Corticium microsclerotia*. A. Early field and transit lesions. B. Transit lesions similar to anthracnose. C. Late field lesions; sclerotia beginning to appear.

pened to be directly beneath or close to a pod lesion always show a discoloration.

THE FUNGUS

Taxonomy.—The vegetative stage of the fungus has been identified as *Rhizoctonia microsclerotia* Matz, which was described (5) on fig. Burger (2), Stevens (12) and Weber (13) reported the fungus on fig as distinct from any other species of the genus, while Chupp (3) considered it as a possible form of *R. solani* Kühn, and Simon-Thomas (11) stated that it was a strain of *R. solani.* Shaw (10) described several diseases apparently caused by *Rhizoctonia* spp., one of which was similar to this disease, except that the fungus causing it produced black sclerotia.

Studies of web-blight in nature, and the fungus in pure culture have shown it to be distinct from *R. solani* Kühn on potato, cabbage, bean, tomato and pine seedlings, *R. zeae* Voorhees on corn and pine seedlings and from *Corticium stevensii* Burt, the cause of thread blight on fig, pear, tung, and pecan. Characteristics of the fungus are indistinguishable on the wide range of hosts already mentioned. The size and color of the sclerotia characterize the fungus in nature, and mycelia and sclerotia aid in its identity in culture.

Basidiospores produced by the fungus on beans and figs were germinated and grown on 2 per cent potato-dextrose agar. On this medium the mycelia and sclerotia produced by the basidiospore cultures were indistinguishable from each other and from cultures produced by either sclerotia or mycelium obtained from the same hosts (Fig. 4). They did, however, differ from cultures obtained from basidiospores of either *Corticium vagum* B. and C. or *Corticium stevensii* Burt. Since the fungus is distinct from related species and has not previously been recognized by a basidiospore stage, the following binomial is proposed:

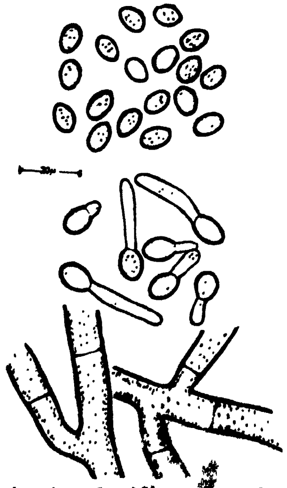

FIG. 4. Mature and germinating basidiospores of *Corticium microsclerotia* and hyphae of the fungus from culture, showing typical branching and septation.

Corticium microsclerotia (Matz), n. comb.

Syn. *Rhizoctonia microsclerotia* Matz.

Phytopath. 7: 110–117. 1917.

The original description of the fungus is as follows:

> *Rhizoctonia microsclerotia* n. sp.—Sclerotia superficial, small 0.2 to 0.5 mm. in diameter, white when young, brown to dark brown at maturity, nearly homogeneous in structure and color, sub-globose, free from tufted mycelium, not smooth, usually single, sometimes conglomerated. Vegetative hyphae 6 to 8 μ wide, first hyaline and granular, brown and more or less empty at maturity, septate.
>
> Hab.—On living leaves, branches and fruit of the cultivated fig. *Ficus carica*, Gainesville, Florida, U. S. A.

This should be amended to include the following, excepting parentheses, (sclerotia superficial), seated on fungous hyphal strands growing over surfaces of host, 80–300 × 80–600 μ, averaging 200 × 350 μ, (sub-globose) to elongate, subepidermal hyphae 4 to 6 μ wide, hyaline, septate.

Basidiospores—oval, hyaline, thin-walled, showing point of attachment, 5–6 × 9–11 μ.

Hab.—Certain other woody perennials, beans, and numerous other annuals. Type locality, LaCrosse, Florida; original collection deposited in Florida Agricultural Experiment Station Herbarium, Specimen No. 7850.

Morphology.—The fungus produces 2 types of mycelium; the surface and subepidermal. The epidermis is penetrated directly by the surface hyphae. The subepidermal hyphae are more restricted in diameter and grow into the intercellular spaces, where they kill the host cells. The superficial hyphae, which are about twice as large as the subepidermal hyphae grow rapidly, and spread out fan-wise, from a point of contact or from the petiole, over the leaf blade. When numerous they hold plant parts together as though wrapped with spiderweb. The relatively small sclerotia are very distinctly characteristic of the fungus. Their relative size was demonstrated by sifting 5 cc. of field collections of them through a series of screens. All the sclerotia passed the 10-, 20- and 40-mesh screens. Groups of sclerotia held together in clusters by the fungous hyphae failed to pass through the 60-mesh screen, and "giant," or irregular-shape, sclerotia failed to pass through the 80-mesh and 100-mesh screens. Almost a pure collection of sclerotia, very uniform in size, shape, and color, passed through the 100-mesh screen (Fig. 7, top). The sclerotia constitute the stage of the fungus by which it usually is disseminated locally and by which it survives periods when host plants are lacking.

The wedge- to oval-shape basidia, produced on the hyphae closely attached to the epidermis of the host, produce 4 basidiospores on as many sterigmata. The basidiospores are hyaline, nonseptate, oval, slightly irregular, showing point of attachment, measuring 5–6 × 9–11 μ.

Physiology.—The fungus grows readily in complete darkness or continuous, intermittent, or subdued light and covers the surface of a Petri dish in 24 to 36 hours at 80° to 85° F. on most of the artificial media used in the laboratory. The sclerotia scattered directly from the host plant on the surface of potato-agar plates show 100 per cent germination in 12 hours. Sclerotia produced in culture are tawny to dark brown, irregular in shape and size, more or less flattened, up to 1 cm. in diameter, and do not resemble those produced on the host plants. The fungus isolated from fig and grown in culture resembles in every way the fungus isolated from bean.

Isolations of *Corticium microsclerotia* from fig, bean, cowpea, and varnish tree; *Rhizoctonia zeae* Voorh. from pine seedlings; and *C. vagum* B. and C. from potato tubers, cabbage, tomato fruits, and pine seedlings grown in parallel on steamed bean pods showed that those of the first group of cultures were indistinguishable, and that all produced large irregular brown sclerotia on the bean pods after 2 weeks. *R. zeae* was different and produced small scattered sclerotia. The third group were all alike and differed from the first and second groups by the production of smaller, darker brown sclerotia, attached to the walls of the test tubes.

Observations on web-blight in the field during periods of its maximum destructiveness, verified by controlled inoculation experiments and correlated with pure-culture study, indicate that it is favored by relatively high temperatures. Studies on the influence of temperature upon the fungus included isolations of *Corticium microsclerotia* from fig, bean, cowpea, and varnish tree, and *C. vagum* from cabbage, as a check. All the medium was prepared simultaneously, adjusted to a reaction of pH 6, poured into Petri-dishes, inoculated in quadruplicate by transfers of the respective fungi, and incubated in the dark in constant temperature chambers ranging from 8° to 36° C. The effect of the temperature on the growth of each of the cultures was determined by averaging the measurements of the diameter of the colonies during a 60-hour period. The results of this test are shown in table 2.

TABLE 2.—*Comparative rate of growth in centimeters of isolations of Corticium microsclerotia with C. vagum from cabbage on potato-dextrose agar at indicated temperatures*

Temperature in °C.	Average diameter in cm. of the colonies from				
	Bean	Fig	Cowpea	Varnish tree	(Check) Cabbage
8	.0	.0	.0	.0	.0
15	.5	.5	.5	.5	2.5
17	2.2	2.1	2.0	2.0	5.9
20	2.8	3.8	2.4	3.8	7.3
23	4.1	5.6	3.5	4.5	8.4
26	7.0	8.0	7.1	6.5	*9.0*
29	*7.2*	*8.6*	*7.5*	*7.1*	8.0
32	3.9	4.3	3.5	3.4	7.4
33.5	.0	.0	.0	.0	2.9
36	.0	.0	.0	.0	.0

The temperatures above 24° C. were constant within a degree, while those below that point varied ± 1° C. The growth of each of the 4 replications of the strains of the organisms, excepting that from cabbage, was uniform and quite comparable between replications, while the cabbage fungus grew much faster and within a greater range of temperatures. The cardinal temperatures of the isolates of *Corticium microsclerotia* were 15°, 29°, 33° C. (Fig. 5, A), which is evidence indicating their common identity.

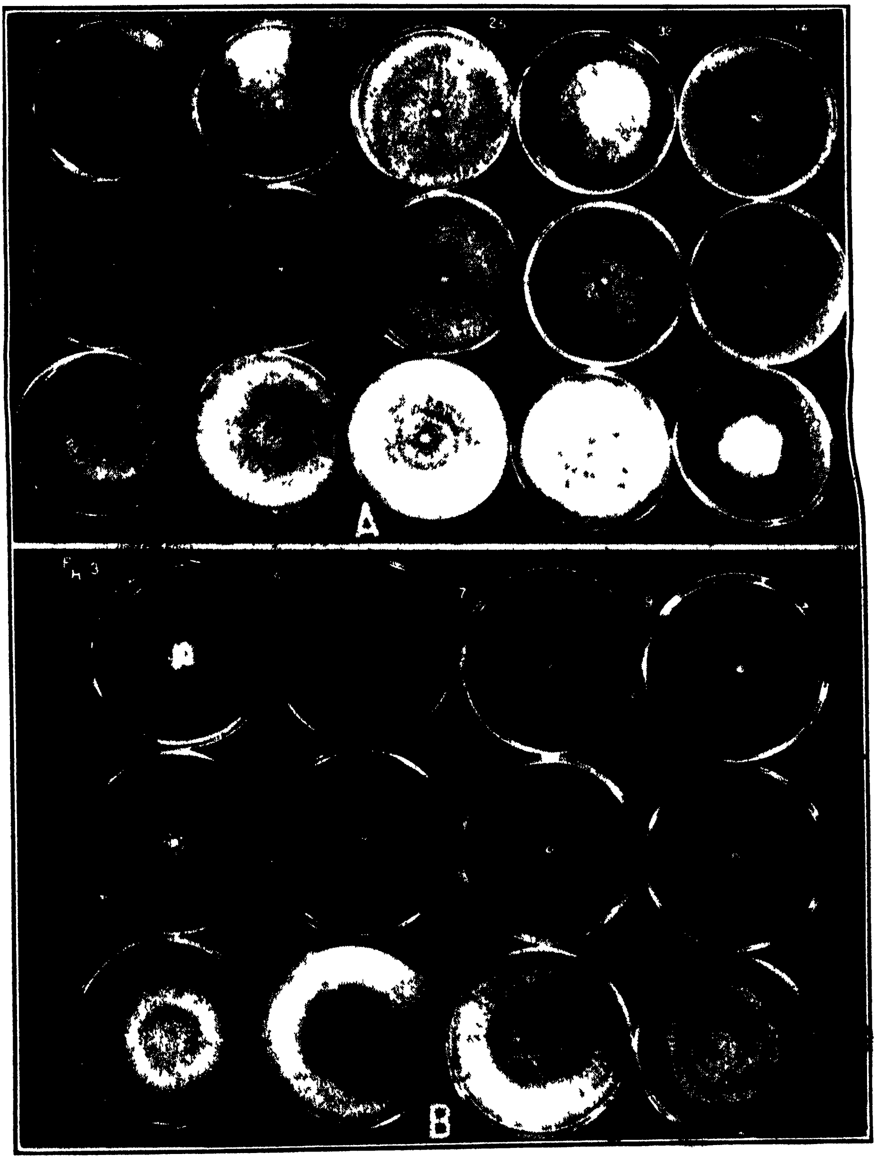

FIG. 5. Pure cultures of *Corticium microsclerotia* isolated from fig (top) and bean (center) and *C. vagum* isolated from cabbage (bottom), respectively, in each photograph, grown in parallel series on 2 per cent potato-dextrose agar at temperatures (A) and hydrogen-ion concentrations (B), indicated.

The same isolates were used in studies on effect of hydrogen-ion reaction of medium upon rate of growth. A 2 per cent potato-dextrose agar prepared in the usual way was neutralized, divided, and, with the aid of a Beckman pH meter, adjusted to a pH series 3, 4, 5, 6, 7, 8, and 9 by adding various amounts of 1/10 normal NaOH or HCl. Plates were inoculated and incubated for 60 hours in the dark at 28° C. (Table 3).

TABLE 3.—*Final pH of inoculated and noninoculated agar plates and comparative rate of growth in centimeters of isolates of Corticium microsclerotia with C. vagum from cabbage on potato agar in 60 hours at indicated hydrogen-ion concentrations*

Host	Hydrogen-ion concentration of medium of noninoculated and inoculated poured plates and growth of fungus in centimeters							
	Original pH	3.	4.	5.	6.	7.	8.	9.
Bean	Checks pH	3.2	4.3	5.5	6.2	7.2	7.7	8.2
	Cultures pH	3.5	6.0	6.6	6.7	6.7	6.6	6.8
	Growth mm.	2.6	4.1	5.3	6.3	5.3	4.9	3.9
Fig	Checks pH	3.2	4.5	5.6	6.2	7.1	7.7	8.1
	Cultures pH	3.5	6.0	6.3	6.6	6.5	6.4	6.5
	Growth mm.	1.7	4.2	6.3	6.4	5.6	4.1	3.9
Cowpea	Checks pH	3.3	4.5	5.6	6.2	7.3	7.9	8.2
	Cultures pH	3.6	6.0	6.4	6.6	6.7	6.7	6.8
	Growth mm.	1.9	4.2	6.3	6.9	6.6	5.4	4.1
Varnish tree	Checks pH	3.3	4.5	5.6	6.3	7.4	7.8	8.2
	Cultures pH	3.3	6.3	6.3	6.4	6.5	6.6	6.8
	Growth mm.	2.9	4.4	6.9	7.6	7.1	5.6	4.6
Cabbage	Checks pH	3.2	4.4	5.6	6.3	7.2	7.6	7.9
	Cultures pH	3.7	6.4	6.5	6.6	6.7	6.7	6.8
	Growth mm.	6.7	8.1	9.0	9.0	8.9	8.6	7.6

During the 60-hour period the noninoculated plates, designated as checks, adjusted to pH 3–4–5–6 and 7, increased in alkalinity, while those adjusted to pH 8 and 9 increased in acidity. All of the inoculated plates changed toward neutrality, except for those adjusted at pH 7, which became more acid.

Corticium vagum from cabbage grew much faster than any of the others and at each extreme of the range showed a greater colony diameter than was developed by the others at the optimum reaction (Fig. 5, B). The other 4 isolations grew at almost equal rates at the different pH concentrations with some growth at pH 3, optimum slightly above pH 6 and some growth at pH 9, indicating the 4 isolations were similar.

Pathogenicity.—The fungus was obtained in culture from surface hyphae, sclerotia, infected host tissue, and basidiospores. These cultures were indistinguishable one from the other, and inoculations showed them to be highly pathogenic.

Thoroughly wet plants about 6 inches tall were inoculated by sprinkling sclerotia over the surface of the leaf blades, set in a humidity chamber for 24 hours, and then placed on a greenhouse bench.

TABLE 4.—*Results of inoculation experiments with Corticium microsclerotia from bean, Gainesville, Florida, 1937*

Plants used	Number inoculated	Incub. period hours	Number diseased	Number checks	Number checks diseased
Tomato	10	48	10	4	0
Beet	10	48	10	4	0
Carrot	10	60	10	4	0
Eggplant	10	48	10	4	0
Cucumber	10	24	10	4	0
Cantaloupe	10	24	10	4	0
Watermelon	10	60	10	4	0
Snap bean	10	24	10	4	0
Lima bean	10	24	10	4	0
Lettuce	10	—	0	4	0

The disease appeared within 3 days after inoculation on all plants, except lettuce. Bean plants were most susceptible and showed the most infection, followed by cucumber and cantaloupe, within a given period; while tomato, beet, and eggplant required a longer time. Carrot and watermelon developed infection more slowly, and none appeared on the inoculated lettuce plants (Fig. 6, B). The fungus was reisolated from the diseased plants and the isolations compared favorably with the original culture of the fungus.

Since some variation was observed in the apparent severity of the disease among the plants infected when the inoculum was obtained from pure cultures, a series of inoculations were made in which the inoculum was obtained from snap beans, from Florahome and Sanford, Japanese varnish tree from Gainesville, and Lima beans from LaCrosse. The plants, about 8 weeks old, 6 to 10 inches tall, and in thrifty growing condition, were inoculated by placing small squares of the agar culture, bearing only mycelium, near the terminal bud of the plants. The surrounding foliage was then sprinkled, cupped, and fastened to form an ideal humidity chamber around the tender growing tip.

TABLE 5.—*Plants inoculated with mycelium of Corticium microsclerotia from pure cultures isolated from different geographical locations and hosts*

Plants inoculated	Number inoculated				Number diseased			
	(a)[a]	(b)	(c)	(d)	(a)	(b)	(c)	(d)
Eggplant	4	4	4	4	4	4	4	4
Pepper	4	4	4	4	4	4	4	4
Tomato	4	4	4	4	4	3	4	4
Lettuce	4	4	4	4	1	2	2	1
Beet	4	4	4	4	4	4	4	4
Carrot	4	4	4	4	4	3	3	3
Cantaloupe	4	4	4	4	4	4	4	4
Cabbage	4	4	4	4	2	1	1	2
Lima bean	4	4	4	4	4	4	4	4
Snap bean	4	4	4	4	4	4	4	4
Varnish tree	4	4	4	4	4	4	4	4

[a] Isolated from (a) Varnish tree, Gainesville; (b) Snap beans, Sanford; (c) Snap beans, Florahome; and (d) Lima beans, LaCrosse.

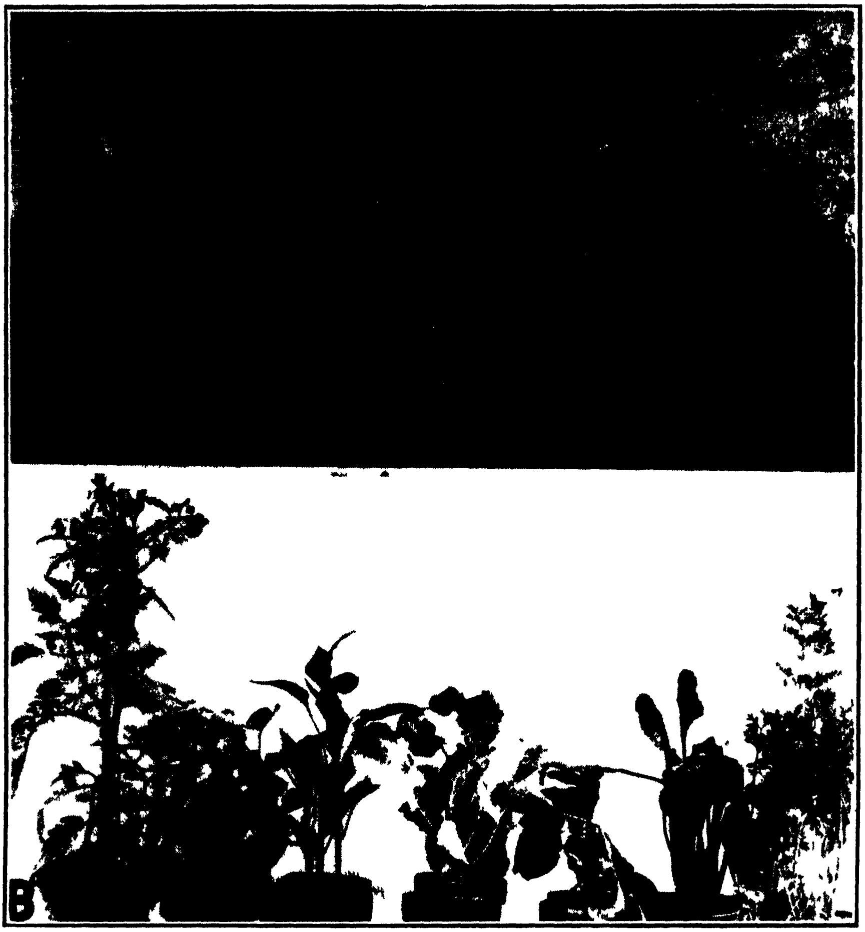

FIG. 6. *Corticium microsclerotia* growing from infected to non-infected leaves in contact showing hyphae and sclerotia. × 3. B. Artificially inoculated tomato, cantaloupe, pepper, cabbage, eggplant, beet, and carrot plants all susceptible to the disease.

All of the plants inoculated with the fungus became infected and there was little variation in the disease as it occurred on a plant variety. However, considerable variation was noted among the varieties. None of the checks became infected. Lettuce and cabbage, although infected, showed the effects of the parasite the least, while tomato and carrot showed some disease-free plants, which was probably because of the looser type of foliage that retarded growth of the fungus. There appeared to be no difference in virulence of the different isolates. Other inoculations were made during high summer temperatures by fastening sclerotia-bearing, diseased leaf tissue of Varnish tree to tender foliage of various perennial woody plants. Results of these tests are shown in table 6.

Infection took place only on Varnish tree and on fig, the latter previously reported susceptible to attacks by this fungus.

TABLE 6.—*Results of inoculations made with Corticium microsclerotia isolated from Varnish tree*

Plant name	Number inoculated	Number diseased	Number checks	Number of diseased checks
Avocado (*Persia drymifolia* C. and S.)	6	0	2	0
Surinam (*Eugenia uniflora* L.)	4	0	2	0
Guava (*Feijoa sellowiana* Berg.)	5	0	2	0
Persimmon (*Diospyrus montana* Roxb.)	4	0	2	0
Sour Orange (*Citrus aurantium* L.)	6	0	2	0
Fig (*Ficus carica* L.)	5	5	2	0
Varnish Tree (*Firmiana platanifolia* (L.) R.Br.)	8	5	2	0

FUNGUS AND HOST RELATIONS

The disease always has been observed in the State during the summer rainy period when the daily maximum temperatures were about 90° F. and a minimum of about 70° F. It has been found to destroy in many instances the late spring crop in June, when the summer rains began early, and the early fall crops in November, when the rainy season was prolonged.

The fungus survives from season to season on plant débris in cultivated fields, along undisturbed fence rows and ditches, and on living plants. The disease has not always been found in these locations before it appeared on the cultivated plants, but it has always been found there during the time when it was severe in the fields. The small, light-weight sclerotia are easily detached and scattered from the diseased plants. Following infection the disease may increase in severity at which time sclerotia are produced in great numbers in very short periods under favorable environmental conditions. The entire surface of the leaves, petioles, and stems have been observed covered with sclerotia, which give the appearance of a coarse brown dust. They are produced also on mycelium growing out to 3 inches from a diseased plant on clods of soil, plant débris, and pieces of decaying wood. Under controlled experiments sclerotia appeared 3 days after inoculation. The prevalence of the disease is affected very little by the age of the plants. Young succulent plants are easily killed and usually disintegrate before many sclerotia are produced, while older, woody plants usually remain standing, even though completely defoliated and often dead.

Wind, rain, running water, and heavy dews are the natural means of dissemination. The sclerotia also are scattered by mechanical means during cultivation, by animals, field implements, and pickers. Large, bushy plants, which retain longer the humidity resulting from dew and rain, favor the development of epidemics of web-blight.

Hyphae a few millimeters in length soon developed from sclerotia kept in a humid atmosphere. With favorable temperature and humidity a profuse branching of the hyphae followed their contact with the host tissue. The tips of the hyphae penetrated directly into the epidermis of the host plant during the night or early morning before the plants became dry.

When the plants were dry, little or no surface growth appeared on young infections. The development of the fungus in older infections was much less affected by humidity. The points of entry showed no correlation with any known environmental factor; and the penetration appeared to be through a puncture slightly smaller in diameter than were the hyphae. Penetration was not observed through stomatal openings nor were appressoria formed.

The shortest time required from inoculation of host to production of viable sclerotia was 3 days, under conditions favorable for the development of the fungus, and 5 to 6 days under normal field conditions. In the inoculation experiments symptoms of the disease appeared on bean plants quicker and more distinctly than on any other plants.

The fungus survives from season to season in the form of sclerotia and as vegetative mycelium attached to or imbedded in dead host material. In laboratory tests it remained viable for 10 months in culture dishes at room temperature. Sclerotia collected in the field on diseased bean plants and held for various lengths of time and under various conditions in the laboratory, were tested for viability by planting them on hard-agar plates.

TABLE 7.—*Percentage of sclerotia of Corticium microsclerotia germinating on hard agar after being removed from the indicated stations on the 15th of each month*

Percentage germination of sclerotia at indicated stations

Tested 15th of	On host in bag			In vials			
	Refrig. 10° C.	Room temp.	Soil surface	Refrig.		Room temp.	
				10° C.	4° C.	Corked	Open
July	100	100	100	100	100	100	100
Aug.	100	100	100	100	100	100	100
Sept.	100	100	50	100	100	100	100
Oct.	100	100	5	100	100	100	100
Nov.	100	100	0	100	100	90	100
Dec.	100	100	0	100	100	50	75
Jan.	100	100	0	100	40	25	90
Feb.	100	100	0	100	50	15	30
Mar.	100	50	0	100	60	5	25
Apr.	100	10	0	100	50	10	10
May	100	0	0	100	15	10	0
June	100	0	0	100	15	0	3
July	100	0	0	100	20	0	0

It may be seen that the viability of the sclerotia, perfect at first, began to decrease after several months, except at 10° C. Diseased material, consisting of bean leaves and stems, matted together by wefts of the surface hyphal strands and detached sclerotia, kept at 10° C., have been plated out on hard agar each month and after a year continued to show vigorous growth when placed on the media at room temperature. The same type of material kept in paper bags on the shelf in the laboratory ceased to show growth after about 9 months.

Stained sections of early infected tissue show both superficial and internal hyphae. The former is greater in diameter, slightly pigmented, and

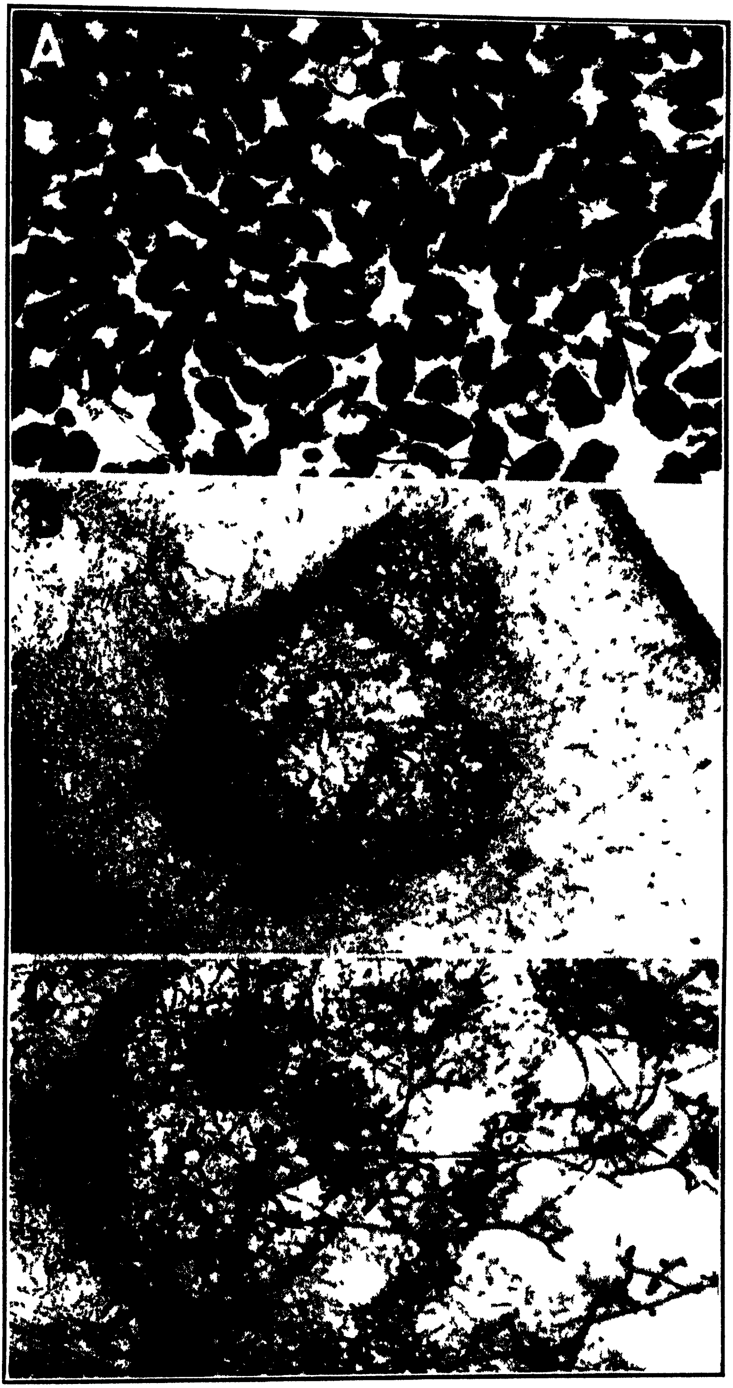

FIG. 7. A. Sclerotia of *Corticium microsclerotia*. ×27. B. Invaded area of leaf 12 hours after inoculation. ×27. C. Superficial hyphae developed from sclerotium in 24 hours. ×90.

grows more rapidly. The internal hyphae are small and hyaline and ramify the tissue in all directions, their extremities coinciding with the dark band that borders the invaded area (Fig. 7, B). These hyphae are very definitely limited in their extension by the larger veins in the leaf blades, resulting in one or more angular sides to the young infection spot. The extremities of the superficial hyphae apply themselves to the host tissue directly or form a series of thickened, short, dichotomous branches. The internal hyphae are at first intercellular but later become intracellular. In relatively dry weather killed leaves remain intact, while in wet weather they are completely disintegrated. Killed cucumber and eggplant leaves disintegrate more rapidly than bean leaves.

CONTROL

On a basis of observations made at the time of occurrence of web-blight and its relations to weather conditions, it would appear that damage from the disease could be reduced by not planting beans during the period between May 30 and September 1 in fields where the disease occurred previously.

Since Bordeaux mixture has controlled the disease on figs (2, 8, 13), the use of this spray is suggested for beans planted during the summer months. When used, Bordeaux should be applied before the plants begin to bloom. Beans grown in the fall, winter or spring should be alternated with some other crop, especially grasses. To prevent the accumulation of sclerotia, which are easily scattered considerable distances, infected plants should be destroyed as soon as possible after they are abandoned. No resistance in beans has been observed.

SUMMARY

A disease of beans heretofore unreported in the United States is described in detail and web-blight is suggested as a common name. It is a destructive disease of beans in fields and in transit.

The fungus infects perennials, such as *Firmiana platanifolia, Ficus carica, Rubus cuneifolius, Sambucus simpsonii,* in Southeastern United States, India, Japan and the East Indies and such annuals as *Phaseolus vulgaris, Vigna sinensis, Xanthium americanum, Echinochloa colona, Eleusine indica* and others in Puerto Rico and Florida.

Web-blight is caused by the fungus *Rhizoctonia microsclerotia* Matz. described from fig in Florida.

The basidiospore stage of this fungus has been found and is described herewith and designated by the binomial *Corticium microsclerotia* (Matz.) n. comb.

Possible control measures in Florida consist in rotation of crops and the non-cultivation of beans from June 1 to September 1 in fields where previous bean crops have shown the disease.

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STUDIES ON THE SUSCEPTIBILITY OF FORAGE GRASSES TO CEREAL SMUT FUNGI. III. FURTHER DATA CONCERNING TILLETIA LEVIS AND T. TRITICI[1]

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INTRODUCTION

The incidence of *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn on hosts other than *Triticum* spp. has been reported by a number of investigators, and in 1936 the writer reviewed the literature dealing with this subject (6). At that time the results of preliminary investigations of the susceptibility of forage grasses to these two species of *Tilletia* were presented. This work was begun following the discovery of the occurrence of *T. tritici* on crested wheatgrass under field conditions in the State of Washington. It was found that, in addition to crested wheatgrass (*Agropyron cristatum* (L.) Beauv.), slender wheatgrass (*A. pauciflorum* (Schw.) Hitchc.), bearded wheatgrass (*A. subsecundum* (Link) Hitchc.), and meadow barleygrass (*Hordeum nodosum* L.) also were susceptible to *T. tritici* and *T. levis*. Furthermore, there was evidence of marked differences in the resistance and susceptibility of the collections and strains of crested and slender wheatgrass that were tested. However, the preliminary work left many questions open, which subsequent experiments have partially or completely answered, and the data presented herein are the results of these investigations. Special emphasis has been placed upon the problems of the perennial nature of *T. tritici* and *T. levis* mycelium in the perennial grass hosts, the relation of physiologic races of these species to resistance of various selections of forage-grass species, and the secondary effects on the grass hosts caused from infection by *T. tritici* and *T. levis*.

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[1] Grass disease investigations of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Soil Conservation Service, Section of Conservation Nurseries, and the Divisions of Plant Pathology and Agronomy of the Agricultural Experiment Station, State College of Washington.

[2] Associate Pathologist, Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

[3] Grateful acknowledgement is made to the Pullman Unit of the Soil Conservation Service, Section of Conservation Nurseries, for supplying seed of many of the grass species; to Mr. D. E. Stephens, formerly Senior Agronomist in the Division of Cereal Crops and Diseases, Bureau of Plant Industry, for supplying seed of about 40 selections of crested wheatgrass; to Dr. C. S. Holton, Assoc. Pathologist, Division of Cereal Crops and Diseases for supplying inoculum of all the races of *Tilletia levis* and *T. tritici* used and seed of differential wheat varieties; and Dr. F. D. Heald, Head of the Department of Plant Pathology, State College of Washington, for critical review of the manuscript of this paper.

MATERIALS AND METHODS

In these experiments the grass seed was inoculated either by shaking the seed and spores together in a glass vial or an envelope, or by Zade's partial vacuum method elaborated by Allison (1). For the latter method the inoculum was in the form of an aqueous suspension of spores. The inoculated seed was sown in 5- or 6-ft. rows in the field, either in the spring or fall, instead of seeding in pots or flats of soil maintained at optimum temperature of 10–14° C. and planting the seedlings in the greenhouse, as was done in the preliminary work.

Percentages of infection in the grasses were calculated on the basis of plant counts, rather than by head counts. In this connection a difficulty is encountered in grass-smut studies that is not usually experienced in the cereals, in that the perennial grasses continue to produce culms throughout a major portion of the growing season. In some instances by the time the last culms of the season are produced, the first have either matured or broken off, or the heads have shattered. For this reason it is desirable to use plant counts rather than head counts if definite percentages of infection are to be calculated. The percentages of smut in the wheat inoculations were calculated by head counts, as usual.

HOST RANGE STUDIES

Up to the present writing the following grasses and cereals (in addition to wheat) have been shown to be susceptible to either or both *Tilletia tritici* and *T. levis: Agropyron cristatum* (6), *A. pauciflorum* (6), *A. subsecundum* (6), *Aegilops ventricosa* Tausch. (8, 12), *Hordeum nodosum* L. (6), *Lolium multiflorum* Lam. (4), *L. perenne* L. (4), *Secale cereale* L. (7, 5, 10, 8, 2, 16, 11).

During 1935–37 collections of the following grasses were tested for susceptibility to *T. levis* and *T. tritici: Agropyron caninum* (L.) Beauv., *A. cristatum*, *A. elongatum* (Host) Beauv., *A. inerme* (Scribn. & Smith) Rydb., *A. pauciflorum*, *A. pungens* (Pers.) Roem. and Schult., *A. repens* (L.) Beauv., *A. sibiricum* (Willd.) Beauv., *A. smithii* Rydb., *A. spicatum* (Pursh.) Scribn.

and Smith, *A. subsecundum, A. trichophorum* (Link) Richt., *Hordeum bulbosum* L., *H. murinum* L., *H. gussoneanum* Parl., *H. nodosum, Lolium perenne, Sitanion hystrix* (Nutt.) J. G. Smith, and *S. jubatum* J. G. Smith. A mixture of 4 of the most virulent races of the two smut species was used for inoculum.[4]

As a result of these inoculations the following species were established as new hosts to *Tilletia levis* and *T. tritici: Agropyron inerme, A. spicatum, A. trichophorum,* and *Sitanion jubatum* (Fig. 1). Infection was also obtained in varying quantities on *A. cristatum, A. pauciflorum,* and *A. subsecundum,* which were reported in the preliminary paper (6).

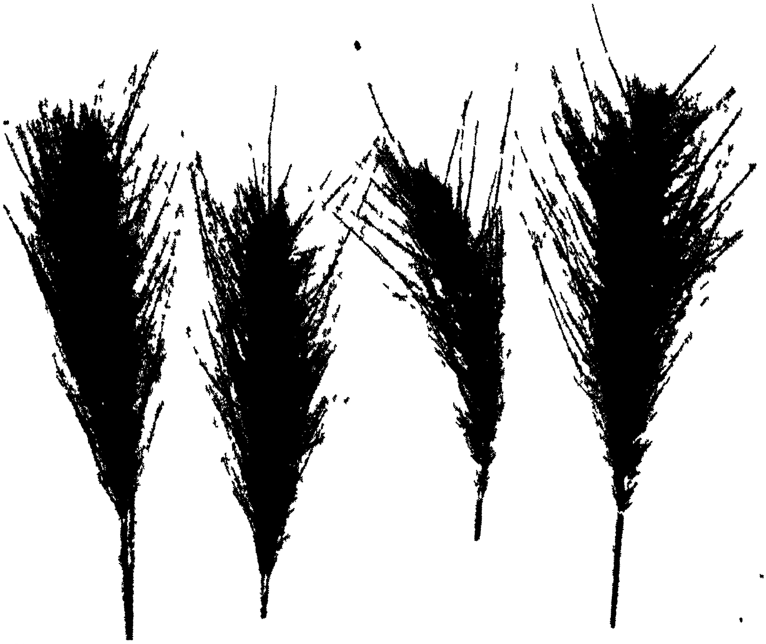

FIG. 1. Wheat bunt (*Tilletia tritici*) on *Sitanion jubatum.* × about ⅜.

COMPARATIVE VIRULENCE OF RACES OF TILLETIA LEVIS AND T. TRITICI ON SELECTIONS OF SLENDER, CRESTED, AND BEARDED WHEATGRASS

Experiments in 1936

As already stated the grass species tested during 1935–37 for susceptibility to wheat bunt were inoculated with a mixture of 4 physiologic races of *Tilletia levis* (1 race) and *T. tritici* (3 races). Where infection resulted from inoculation with this mixture there was no way of determining whether one or more than one race had been responsible for the infection except to inoculate sets of differential wheat varieties with inoculum obtained from the

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[4] This inoculum was supplied through the courtesy of C. S. Holton (see footnote 3) and consisted of races T–8, T–10, and T–11 of *Tilletia tritici*, and race L–8 of *T. levis*, according to the recent classification by Rodenhiser and Holton (15).

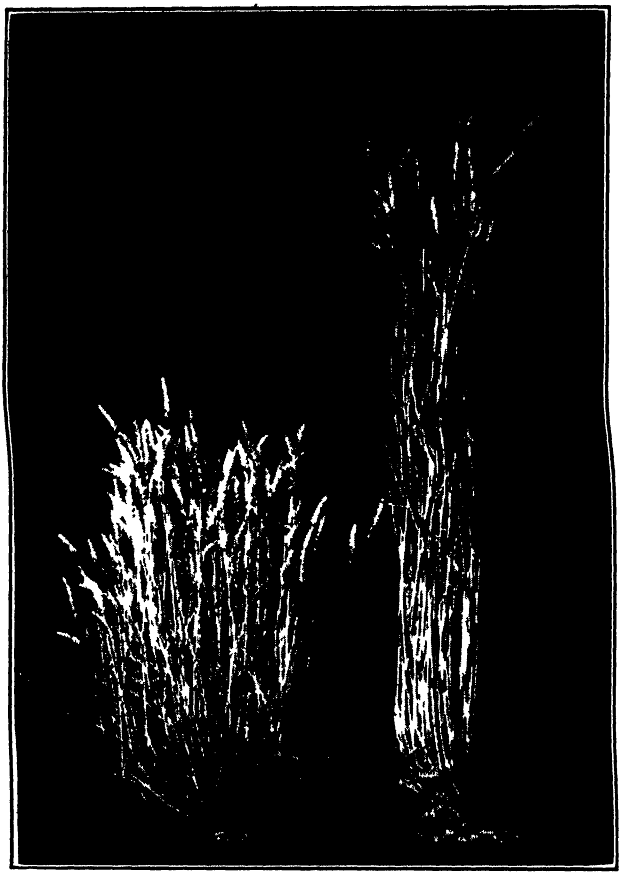

FIG. 2. Bunted (*Tilletia tritici*) and normal (on right) plants of crested wheatgrass, showing the effect of the infection on growth habit. Both plants are of the same selection and grew side by side in same row. × about ⅐.

grasses. This might also give a clue as to whether any one of the 4 races was especially virulent on the grasses infected.

Accordingly, from the selections of *Agropyron subsecundum*, *A. cristatum*, and *A. pauciflorum*, which were smutted in 1935 as a result of artificial inoculation, 10 were selected for a source of inoculum for sets of differential wheat varieties. The smut balls from each smutted selection were pulverized and the spores used to inoculate a set of differentials.[5]

The results of the inoculation of winter wheat differentials are presented in table 1. It appears from these data that all of the physiologic races in the original mixture used for inoculum reappeared when the smut obtained from the grasses was inoculated to the wheat differentials. However, except

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[5] In the present investigations the following wheat varieties have been used as differential hosts: Hybrid 128 (C. I. 4512), Turkey (C. I. 6175), Ridit (C. I. 6703), Oro (C. I. 8220), Albit (C. I. 8275), Martin (C. I. 4463), Hohenheimer (C. I. 11458), White Odessa (C. I. 4655), Hussar (C. I. 4843), Ulka (C. I. 11478), Marquis (C. I. 3641), and Canus (C. I. 11637).

TABLE 1.—*Results of inoculations of winter wheat differentials with Tilletia levis and T. tritici from wheat grasses*

Winter wheat differential	*Agropyron subsecundum* No. 12				*Agropyron cristatum* No. 28				*Agropyron cristatum* No. 44				*Agropyron pauciflorum* No. 61				*Agropyron pauciflorum* No. 62			
	Heads	Smutted	Smut	Phys. Races[a]	Heads	Smutted	Smut	Phys. Race	Heads	Smutted	Smut	Phys. Race	Heads	Smutted	Smut	Phys. Race	Heads	Smutted	Smut	Phys. Race
	No.	*No.*	*Per cent*		*No.*	*No.*	*Per cent*		*No.*	*No.*	*Per cent*		*No.*	*No.*	*Per cent*		*No.*	*No.*	*Per cent*	
Hybrid 128	109	93	85		59	29	49		148	130	88		152	136	89		122	96	79	
Turkey	179	2	1		211	161	76		260	236	91		243	198	81		347	323	93	
Ridit	193	0	0		126	6	5		180	6	3		200	31	15		226	37	16	
Oro	208	1	0		174	6	3		232	21	9		204	193	95	L–8	221	139	63	L–8
Albit	107	0	0		203	135	66	T–8	181	116	64	T–8	222	180	81	T–8	129	0	0	
Martin	136	0	0		167	0	0		175	8	5		257	3	1		171	1	0	
Hohenheimer	38	5	13	T–10	53	0	0		117	0	0		71	0	0		53	0	0	
White Odessa	141	0	0		259	188	73		248	195	79		186	133	71		186	25	13	
Hussar	198	0	0		176	62	36		204	40	20		156	84	54		162	0	0	
	Agropyron pauciflorum No. 71				*Agropyron pauciflorum* No. 75				*Agropyron pauciflorum* No. 84				*Agropyron pauciflorum* No. 10				*Agropyron pauciflorum* No. 6			
Hybrid 128	182	139	76		155	150	97		163	127	78		83	77	93		84	73	87	
Turkey	320	260	81		249	145	58		211	90	43		120	23	19		135	120	89	
Ridit	189	27	14		144	4	28		240	13	5		179	37	21		161	44	27	T–11
Oro	245	30	12		237	12	5		256	12	5		205	62	30	L–8	205	10	5	
Albit	211	130	62	T–8	155	138	89	T–8	183	103	56	T–8	55	2	4		99	0	0	
Martin	164	0	0		275	136	49		275	196	71		164	77	47	T–8	166	0	0	
Hohenheimer	96	0	0		61	0	0		169	0	0		67	0	0		169	0	0	
White Odessa	261	91	35		210	84	40		198	56	28		155	3	2		118	0	0	
Hussar	185	50	27		181	30	17		217	35	16		162	0	0		166	0	0	

[a] It should be remembered that these grasses from which inoculum was taken had been inoculated with a mixture of 4 physiologic races (see footnote 4). Physiologic race, as indicated in this table, refers to the race or races separated from the original mixture by a selective action on the part of the various grasses.

for two instances, (*Agropyron pauciflorum* No. 10, and No. 61) only one race could be detected on any one grass selection. From these results it also appears that race T–8 is the most virulent of the races used, since it reappeared in 7 of the 10 trials. Physiologic race L–8 reappeared 3 times, twice in conjunction with race T–8. The identification of these races is in accordance

TABLE 2.—*Comparative virulence of 19 races of Tilletia levis and T. tritici on selections of Agropyron statum and A. pauciflorum*

Races		*Tilletia levis*								
Grass elections	Acc. no.	L–1	L–2	L–3	L–4	L–5	L–6	L–7	L–8	
		Percentage of infection, based upon number of plants								
cristatum	28	0	50	20	33	50	14	50	40	*Specific*
"	29	50	0	0	0	0	0	0	0	*index of*
"	32	0	0	50	0	33	0	0	0	*virulence*
"	36	0	0	25	14	10	33	0	0	
"	37	25	33	0	57	0	0	33	33	
"	77	0	28	0	67	0	50	33	17	*T. levis*
"	199	0	0	0	0	0	25	0	17	
"	203	0	33	50	0	0	14	0	17	
"	207	0	0	14	25	20	20	29	50	
"	209	11	14	15	13	0	25	0	10	
"	210	8	0	0	6	24	7	12	0	
"	214	25	30	43	0	40	43	22	50	
"	215	17	0	33	0	0	0	0	0	
"	222	25	14	13	20	0	29	6	0	
"	233	25	30	50	11	33	19	17	0	
"	234	9	8	36	10	7	13	8	8	
"	287	24	18	44	30	38	54	33	11	
"	256	18	18	6	14	10	9	0	17	
"	304	13	5	35	15	33	6	17	13	
"	305	20	9	0	33	11	28	10	8	
o. sel. smutted		13	13	14	14	12	16	12	13	218.0
ve. per cent smut in 20 sel.		13.50	14.50	21.70	17.40	15.45	18.55	13.50	14.55	
dex of virulence[a]		175.5	188.5	303.8	243.6	185.4	296.8	162.0	189.1	
pauciflorum	62	13	28	25	23	16	24	19	8	
"	63	0	0	0	0	0	0	0	0	
"	65	0	0	0	0	0	0	0	0	
"	67	0	0	0	0	0	0	0	0	
"	68	16	30	6	20	25	12	13	23	
"	69	0	0	7	6	20	0	17	7	
"	74	0	0	0	0	0	0	0	0	
"	75	13	28	23	33	0	0	0	0	
"	83	0	0	0	0	0	0	0	0	
"	84	13	8	0	40	25	6	0	0	
"	251	0	0	0	0	0	0	0	0	
"	252	0	0	0	13	0	0	0	0	
"	253	0	0	0	0	0	0	0	0	
"	254	0	0	0	0	14	20	0	17	
"	288	0	40	0	0	0	0	0	0	
"	306	0	0	0	0	0	0	0	0	
o. sel. smutted		4	5	4	6	5	4	3	4	23.8
ve. per cent smut in 16 sel.		3.43	8.37	3.81	8.43	6.25	3.87	3.06	3.43	
dex of virulence		13.7	41.8	15.2	50.5	31.2	15.4	9.1	13.7	

with the latest treatise on physiologic races of *Tilletia levis* and *T. tritici* in the United States (14), although it is realized that some of the infection percentages are not so high as they should be to make positive identification possible. Some of the incongruities may have resulted from a hybrid race having arisen through the use of a mixture of races for inoculum.

TABLE 2—(*Continued*)

Races		*Tilletia tritici*												
Grass selections	Acc. no.	T-1	T-2	T-3	T-4	T-5	T-6	T-7	T-8	T-9	T-10	T-11	Check row	
		Percentage of infection, based upon number of plants												
A. cristatum	28	0	63	33	38	28	100	0	33	0	60	33	0	*Speci.*
"	29	0	0	0	25	0	0	25	0	0	33	0	0	*index*
"	32	50	0	0	25	0	0	0	100	0	50	0	0	*of vir*
"	36	40	22	0	33	0	17	0	28	0	0	0	0	*lence*
"	37	0	25	17	40	0	0	0	33	0	4		0	
"	77	13	17	33	44	0	17	0	0	17	0	14	0	*T. tri*
"	199	0	0	0	0	0	0	0	0	0	0	0	0	*tici*
"	203	0	16	18	0	8	7	23	20	10	14	0	0	
"	207	17	0	0	0	11	13	20	40	0	0	0	0	
"	209	0	20	0	0	7	17	14	0	11	10	0	0	
"	210	0	5	5	10	12	7	8	0	10	0	11	0	
"	214	13	22	45	25	20	0	40	22	20	14	20	0	
"	215	13	14	18	0	0	0	0	0	0	25	33	0	
"	222	0	33	0	0	13	0	0	0	0	0	0	0	
"	233	0	14	0	25	0	0	11	33	17	29	0	0	
"	234	14	18	21	6	13	8	12	13	19	29	0	0	
"	287	0	17	0	30	31	37	29	17	25	32	55	0	
"	256	0	20	0	0	20	8	18	23	16	20	0	0	
"	304	7	22	6	6	17	9	20	7	14	13	16	0	
"	305	20	25	6	36	23	14	33	36	25	21	6	0	
No. sel. smutted		9	16	10	13	12	12	12	13	11	13	8	0	162.1
Av. per cent smut in 20 sel.		9.35	17.65	10.10	17.15	10.15	12.70	12.65	20.25	9.20	17.50	9.40		
Index of virulence[a]		84.1	282.4	101.0	222.9	121.8	152.4	151.8	263.2	101.2	227.5	75.2		
A. pauciflorum	62	13	19	42	29	32	42	27	32	10	52	17	0	
"	63	0	0	0	0	0	0	0	0	0	6	0	0	
"	65	0	0	0	0	0	0	0	0	0	0	0	0	
"	67	0	0	0	0	0	0	0	0	0	0	0	0	
"	68	17	20	0	25	33	14	17	0	24	69	16	0	
"	69	0	0	0	0	6	0	0	17	6	28	13	0	
"	74	0	0	0	0	0	0	0	0	0	0	0	0	
"	75	13	6	23	5	6	0	14	0	0	22	7	0	
"	83	0	0	0	5	0	0	11	20	0	0	0	0	
"	84	17	27	0	0	0	6	6	0	0	0	0	0	
"	251	0	0	0	0	0	0	0	0	0	0	0	0	
"	252	0	0	0	0	0	0	0	0	0	0	0	0	
"	253	0	0	0	0	0	0	0	0	0	0	0	0	
"	254	8	10	7	13	0	0	0	25	17	25	0	0	
"	288	0	0	0	14	29	0	0	0	0	29	0	0	
"	306	0	0	0	0	0	0	25	0	0	25	0	0	
No. sel. smutted		5	5	3	6	5	3	6	4	4	8	4	0	32.3
Av. per cent smut in 16 sel.		4.25	5.12	4.50	5.68	6.62	3.87	6.25	5.87	3.56	16.00	3.31		
Index of virulence		21.2	25.6	13.5	34.0	33.1	11.6	37.5	23.4	14.2	128.0	13.2		

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[a] See footnote 7.

Experiments in 1937

The data from the 1936 experiments give information concerning only 4 of the 19 recognized races (14) of *Tilletia levis* and *T. tritici*. Since inoculum of all of these 19 races was available to the writer,[6] extensive inoculation experiments were begun in the autumn of 1936, using all of these races. Seed of 58 selections and collections of crested wheatgrass and 30 of slender wheatgrass were inoculated with each of the 19 races and seeded in the field. During the subsequent very severe winter all of the crested wheatgrass and most of the slender wheatgrass were winter-killed. In the early spring (1937) the experiment was repeated, less extensively, using 20 crested wheatgrass selections,[6] and 16 of slender wheatgrass. Such an experiment was designed not only to give data concerning the relative virulence of the wheat-smut races, but also to provide a better test of the general varietal resistance and susceptibility of the grass selections than could be gained by inoculating with a mixture of races. The results of these inoculations are presented in table 2.

An analysis of the data in table 2 shows that there is a distinct difference in the relative virulence of the 19 races of *Tilletia levis* and *T. tritici* on selections of crested and slender wheatgrass. On crested wheatgrass such races as L–3, L–6, T–2 and T–8, with an index of virulence[7] of 303.8, 296.8, 282.4, and 263.2, respectively, stand out as distinctly more virulent than certain others, such as T–1, T–3, T–9, and T–11, having an index of virulence of 84.1, 101.0, 101.2, and 75.2, respectively.

On slender wheatgrass the differences in relative virulence are even greater than on crested wheatgrass, especially with regard to race T–10, which, with an index of virulence of 128.0, is several times as virulent as races L–7, T–6, and T–11, whose indices are 9.1, 11.6, and 13.2, respectively.

In comparing the virulence of the two smut species on crested and slender wheatgrass selections, it is seen that on crested wheatgrass, *Tilletia levis*, with a specific index of 218.0, is decidedly stronger than *T. tritici*, the specific index of which is 162.1. However, with a specific index of 23.8, *T. levis* is slightly inferior on slender wheatgrass to *T. tritici* whose specific index on this host is 32.3.

These results from the 1936 inoculations confirmed those of 1935 (Table 1), where races L–8 and T–8 appeared especially virulent.

VARIETAL RESISTANCE AND SUSCEPTIBILITY OF CRESTED WHEATGRASS AND SLENDER WHEATGRASS TO TILLETIA LEVIS AND T. TRITICI

The results of the foregoing experiments on the relative virulence of races of *Tilletia levis* and *T. tritici* on crested and slender wheatgrass selections, although yielding some interesting information on this subject, do, nevertheless, show that even greater differences are to be seen in the reactions of the grass selections to the smut races, than in the relative virulence of the latter.

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[6] See footnote 3.

[7] The index of virulence for each race is the product of the average per cent of smut in the selections inoculated and the number of selections the race was able to infect. Thus, this measure of relative virulence of physiologic races is designed to take into consideration not only the average percentage of infection, which alone is an inadequate measure when a number of host varieties have been used, but also the number of such varieties or selections a given race is capable of infecting.

Reference to table 2 shows that the 20 selections of crested wheatgrass varied in their reaction to *Tilletia levis* and *T. tritici* from highly resistant to all the 19 races to moderate susceptibility to these races. The 16 selections of slender wheatgrass varied from immune to moderately susceptible.

The varietal differences exhibited by these selections of crested and slender wheatgrass in their reaction to races of *Tilletia levis* and *T. tritici* are summarized and analyzed in table 3, which is compiled from the data in table 2. None of the 20 crested wheatgrass selections proved to be absolutely immune from *T. levis,* although Acc. No. 29, 199, and 215, with a susceptibility index[8] of 6.2, 10.5, and 12.3, respectively, are highly resistant. In sharp contrast to these selections are some that are moderately susceptible, such as Acc. No. 28, 214, and 287 whose susceptibility index is 224.8, 221.3, and 252.0, respectively. The reaction of the selections of crested wheatgrass to *T. tritici* races is much the same. To this species Acc. No. 199 appears to be immune. This particular selection is the only one which is immune from or highly resistant to both *T. levis* and *T. tritici.*

Slender wheatgrass, as a species, showed more marked differences in varietal resistance and susceptibility to *Tilletia levis* than did crested wheatgrass. Table 3 shows that 8 of the 16 selections tested appear to be immune from all of the 8 races of *T. levis.* Only two selections (Acc. No. 62 and 68) were moderately susceptible, and the majority were more or less resistant. The reaction to the 11 races of *T. tritici* was somewhat less resistant than to *T. levis,* with the same selections appearing immune or highly resistant. Of outstanding comparative susceptibility is Acc. No. 62. This selection exhibited more or less susceptibility to each of the 11 races of *T. tritici,* had an average of 28.63 per cent infection, and a susceptibility index of 314.9, the highest reaction in the whole experiment.

As a species, crested wheatgrass is more susceptible to wheat bunt than is slender wheatgrass. This is shown by their specific indices of susceptibility (129.2 and 143.3 as compared with 42.8 and 68.3).

On the basis of the results of these varietal tests it seems evident that there is considerable promise in the possibility of control of wheat bunt in slender wheatgrass and crested wheatgrass by the use of resistant varieties. Tables

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[8] In the author's opinion the susceptibility of a host selection to a number of races of a plant pathogen is not accurately measured by percentages of infection alone. The number of races by which it was infected must also be considered: For this reason the "Susceptibility Index" was evolved as a combined qualitative and quantitative measure of the relative susceptibility of the 36 selections of crested wheatgrass and slender wheatgrass to *T. levis* and *T. tritici.* Reference to table 3 will illustrate the value of the susceptibility index as a measure of susceptibility. For instance in table 3 it will be noted that on the basis of the number of races by which they were infected, crested wheatgrass selections Acc. No. 28, 203, and 210 are equally susceptible to *T. tritici,* yet they have widely differing average percentages of infection. Hence, it seems that the product of the number of races capable of infecting a selection by the average percentage of infection by those races should serve as a fair index to the relative susceptibility of selections to a species composed of a number of physiologic races.

data in Table 2)

Species	Acc. no.	*Tilletia levis*					*Tilletia tritici*				
		No. races infecting	Av. per cent infection	Spec. av. per cent inf.	Susc. index[a]	Spec. susc. index	No. races infecting	Av. per cent infection	Spec. av. per cent inf.	Susc. index[a]	Spec. susc. index
A. cristatum	28	7	32.12	16.17	224.8	129.2	8	31.81	13.03	254.4	143.3
"	29	1	6.25		6.2		3	7.54		22.6	
"	32	2	10.37		20.7		4	20.45		81.8	
"	36	4	10.25		41.0		5	12.72		63.6	
"	37	5	22.62		113.1		4	10.45		41.8	
"	77	5	24.37		121.8		7	12.54		87.7	
"	199	2	5.25		10.5		0	0.00		0.0	
"	203	4	14.25		57.0		8	10.54		84.3	
"	207	6	19.75		118.5		5	9.18		45.9	
"	209	6	11.00		66.0		6	7.18		43.0	
"	210	5	7.12		35.6		8	6.18		49.4	
"	214	7	31.62		221.3		10	21.90		219.0	
"	215	2	6.25		12.5		4	9.36		37.4	
"	222	6	12.25		73.5		2	4.18		8.3	
"	233	7	22.00		154.0		6	11.72		70.3	
"	234	8	12.37		98.9		10	13.90		139.0	
"	287	8	31.50		252.0		9	24.81		223.2	
"	256	7	11.50		80.5		7	11.36		79.5	
"	304	8	17.12		119.8		11	12.45		136.9	
"	305	7	14.87		104.0		11	22.27		244.9	
A. pauciflorum	62	8	19.50	5.35	156.0	42.8	11	28.63	6.21	314.9	68.3
"	63	0	0.00		0.0		1	0.54		0.5	
"	65	0	0.00		0.0		0	0.00		0.0	
"	67	0	0.00		0.0		0	0.00		0.0	
"	68	8	18.12		144.9		9	21.36		192.2	
"	69	5	7.12		35.6		5	6.36		31.8	
"	74	0	0.00		0.0		0	0.00		0.0	
"	75	4	12.12		48.4		8	8.72		69.7	
"	83	0	0.00		0.0		3	3.27		9.8	
"	84	5	11.50		57.5		4	5.09		20.3	
"	251	0	0.00		0.0		0	0.00		0.0	
"	252	1	1.62		1.6		0	0.00		0.0	
"	253	0	0.00		0.0		0	0.00		0.0	
"	254	3	6.37		19.1		7	9.54		66.7	
"	288	1	5.00		5.0		3	6.54		19.6	
"	306	0	0.00		0.0		2	4.54		9.0	

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[a] The susceptibility index for each selection is the product of its average percentage of infection and the number of races capable of infecting it.

2 and 3 indicate several selections of each grass whose high resistance or immunity would warrant their use in the cultural control of the wheat-bunt organisms on these forage grasses. If any of these selections should prove to be undesirable agronomically, they could at least serve as resistant parent stock in the breeding of varieties that are at once bunt resistant and also agronomically desirable.

PERENNATION OF TILLETIA LEVIS AND T. TRITICI IN PERENNIAL HOSTS

The interesting and important question concerning the ability of the mycelium of *Tilletia levis* and *T. tritici* to maintain itself in the perennial parts of the grass hosts of these fungi has been included to some extent in the present studies. This possibility has not, of course, concerned cereal pathologists by the very reason of the annual habit of the cereals. With the grasses, however, not only must the possibility be considered; it is of fundamental importance. Crested wheatgrass plants have been known to live for 15 years or more, and other grasses almost as long, and the question naturally arises as to whether or not the smut organisms can maintain themselves in the perennial parts of the host throughout its life.

Perennially infected plants, producing a crop of smut year after year, would be much more important from the standpoint of maintaining the smut organisms and serving as a continual source of inoculum for the seedlings from surrounding healthy plants, than if infection were merely a matter of a year or two in duration.

The nursery plots yielding data for the earlier report of the susceptibility of forage grasses to *Tilletia levis* and *T. tritici* (6) have been useful also in the study of perennation of the smut mycelium, since the individual grass plants were spaced and a 3-year record has been kept of each plant. Thus, over this 3-year period data have been taken concerning 90 bunt-infected plants of crested wheatgrass, slender wheatgrass, bearded wheatgrass, and meadow barleygrass.

Eighty-one per cent of the crested wheatgrass plants and 75 per cent of the slender wheatgrass plants showing smut in 1935 again showed smut in 1936. Data were again taken in 1937 on the same smutted plants. The plant-by-plant records during the 3 seasons (1935–37) are summarized in table 4.

On the basis of the data presented in table 4 it seems that the mycelium of *Tilletia levis* and *T. tritici* can maintain itself in perennial hosts, but not indefinitely, since the number of infected plants grows smaller each year. This is due to, (1) the death of some smut-infected plants, and, probably, (2) the death of the mycelium in other infected plants.

Of the 90 plants of *Agropyron cristatum, A. pauciflorum, A. subsecundum,* and *Hordeum nodosum,* infected with wheat bunt in 1935, 39 were in some manner freed of infection during the 2 years following, 7 died, and 44 still retained the disease in 1937. In two instances plants apparently healthy in 1935 showed smut in 1936, but these did not show infection in 1937. Also,

TABLE 4.—*Summary of records of individual bunted (Tilletia levis and T. tritici) Agropyron and Hordeum plants during 1935–37, inclusive*

Grass species	Total no. smutted plants			No. smutted 1935, smut-free 1936[a]	No. smut-free 1935, smutted 1936	No. smutted 1935, dead 1936	No. smutted 1936, smut-free 1937	No. smutted 1936, dead 1937	No. smut-free 1936, smutted 1937	No. showing smut 1935, 1936, 1937
	1935	1936	1937							
A. cristatum	47	41	26	7	1	0	11	4	0	23
A. pauciflorum	37	28	17	10	1	1	10	1	0	17
A. subsecundum	3	2	1	1	0	0	0	0	0	1
H. nodosum	1	1	0	0	0	0	0	1	0	0
Totals	88	72	44	18	2	1	21	6	0	41

[a] In every case, plants smutted 1935, but smut-free 1936, were also smut free 1937.

in every case, plants that showed smut in 1935 but not in 1936, were still bunt-free in 1937.

In so far as conclusions may be drawn from 3 seasons' observations, it may be said that perennial grasses, infected with *Tilletia levis* and *T. tritici*, may be expected to harbor the mycelium of these smut species in the perennial parts of the host over a period of years. The length of time will probably depend to some extent on the general environmental conditions. Extreme drought or severe winters may kill the infected plants, already weakened. Possibly also, the degree of susceptibility of the infected plants may influence the duration of the perennation of the fungus in the host. The mycelium might be expected to die out gradually in the case of the less-compatible hosts.

ANOTHER INSTANCE OF THE NATURAL OCCURRENCE OF TILLETIA TRITICI ON A FORAGE GRASS

In the spring of 1935 some smut balls were found in a sample of sweet clover seed by Elizabeth McKay Hermann, Seed Analyst at the Washington State College Agricultural Experiment Station, who gave them to the author for investigation. These smut balls obviously were not transformed sweet-clover seeds, but appeared to resemble more the seeds of some grass. The smut balls themselves were closely similar to those produced on crested wheatgrass by *Tilletia tritici.* A comparison of the membrane around the normal caryopsis of crested wheatgrass and that around the smut balls revealed a close microscopic similarity between the two. The spores compared favorably with those of *T. tritici* and the collection was provisionally assigned to this species. However, the very susceptible spring wheat variety, Hard Federation, gave purely negative results when inoculated with this collection of smut. One selection each of slender wheatgrass and crested wheatgrass was also inoculated, with the result that only one infected plant of slender wheatgrass and none of crested wheatgrass appeared. From this one infected slender wheatgrass plant the smut balls were collected and pulverized to make inoculum for the inoculation of a set of winter wheat differential varieties. The seed was sown in the fall of 1936. The results of this test are given in table 5.

TABLE 5.—*Reaction of winter wheat differential varieties to a collection of Tilletia tritici from an unknown grass*

Differential varieties	C. I. No.	No. heads	No. smutted	Percentage smut
Hybrid 128	4512	442	420	95
Ridit	6703	303	133	43
Oro	8220	367	32	8
Albit	8275	397	303	76
Hohenheimer	11458	153	0	0
Hussar	4843	370	100	27

The data in table 5 substantiate the fact that the collection of smut in question morphologically resembles *Tilletia tritici.* The susceptible reaction of Hybrid 128, Ridit, and Albit leaves no doubt that the original collection of smut, found as smut balls in a sweet clover seed sample, was in reality *T. tritici.*

An attempt to establish the race identity of this grass collection of *Tilletia tritici,* using the latest treatise on the subject (14), was not definitely successful. The collection did not seem to conform to any known race of this species, since none has both Albit and Ridit as susceptible varieties. As a further check on this the smut on each of the differentials (except Hybrid 128), which yielded smut in 1937 (*i.e.,* Ridit, Albit, Oro, and Hussar), was inoculated back to sets of winter-wheat differentials, and the seed was sown in Oct., 1937. In addition, the spring wheat differentials Marquis, Ulka, and Canus were inoculated with the smut (from Hybrid 128), and planted in the greenhouse.

The results of these inoculations of winter and spring wheat differentials are presented in table 6. From these data it is seen that the results of the 1937 inoculation are somewhat different from those of 1936. The high percentage of infection on Ridit obtained in 1936 was not even approached in 1937. However, the 1937 inoculations otherwise check closely with those of 1936. The reaction of Ridit for these 2 years is difficult of definite explanation. On the basis of the 1937 results with both the winter and spring-wheat differentials, this collection of *Tilletia tritici,* originally found as smut balls in a sample of sweet clover seed, but apparently from crested wheatgrass, appears to belong to physiologic race T–8, according to Rodenhiser and Holton's recent classification (14).

SOME SECONDARY EFFECTS OF BUNT INFECTION ON CRESTED WHEATGRASS AND SLENDER WHEATGRASS

In the foregoing pages, where the perennation of the mycelium of *Tilletia tritici* and *T. levis* is discussed, reference was made to the gradual dying out of some of the bunt-infected plants of crested wheatgrass and slender wheatgrass. General observations during the three seasons, during which perennation was studied, indicate that the gradual dying out of bunt-infected plants may be expected, since, although table 4 does not show it, it has been very definitely noted that almost all of the infected plants have grown progressively weaker. When perennation data were taken for the 1937 season it was especially noted that the majority of the bunted plants were so devitalized that they were just barely alive and bore only a few very small heads. It is probable that they scarcely survived the very severe winter of 1936–37. Since the uninfected plants in the same plots were very vigorous following the same winter, it appears that bunt-infected crested and slender wheatgrass plants are decidedly predisposed to winter injury. This same phenomenon has been noted in wheat and proved experimentally by Holton and Heald (9) and by Schlehuber (15).

Furthermore, it has been observed, also in wheat, that bunt infection, at least with some races of *Tilletia tritici,* tends to produce stunted plants (3, 13). The writer has consistently observed the same phenomenon in

TABLE 6.—*Reaction of winter and spring wheat differentials to a grass collection of Tilletia tritici propagated on the same differentials*

Differential variety	Differential variety on which original collection was propagated											
	Oro			Hussar			Ridit			Albit		
	Heads in row	Heads smutted	Smut	Heads in row	Heads smutted	Smut	Heads in row	Heads smutted	Smut	Heads in row	Heads smutted	Smut
	No.	*No.*	*Per cent*	*No.*	*No.*	*Per cent*	*No.*	*No.*	*Per cent*	*No.*	*No.*	*Per cent*
Hybrid 128	372	355	95	341	314	92	316	300	95	245	222	91
Ridit	230	12	5	276	15	5	224	17	8	255	40	16
Oro	284	22	8	283	33	12	240	15	6	267	30	11
Albit	199	191	96	238	227	95	312	295	95	213	192	90
Hohenheimer	242	0	0	214	0	0	200	0	0	230	6	3
Hussar	221	186	84	184	135	73	200	167	83	170	145	85
		Hybrid	128									
	Heads in row	Heads smutted	Smut									
	No.	*No.*	*Per cent*									
Marquis	93	59	63									
Ulka	39	21	54									
Canus	151	91	60									

crested wheatgrass affected with bunt, as illustrated in figure 2, and lesser modifications in slender wheatgrass. Such stunted plants are not so vigorous and generally healthy as uninfected plants, and the forage value obviously is lessened.

SUMMARY

The results of additional studies of the susceptibility of forage grasses to *Tilletia levis* and *T. tritici* are given in this paper. Inoculations of various grasses in the tribe Hordeae have added *Agroypron inerme, A. spicatum, A. trichophorum,* and *Sitanion jubatum* to the list of species that may be hosts to the wheat bunt fungi.

Preliminary inoculations of crested and slender wheatgrass selections with a mixture of 4 physiologic races of *Tilletia levis* and *T. tritici,* followed by a later separation of the races from the bunted grass plants, indicated that the races in this mixture differed in their virulence on these grasses. In a more complete inoculation experiment 20 selections of creasted wheatgrass and 16 of slender wheatgrass were each inoculated with each of 8 races of *T. levis* and 11 of *T. tritici.* It was found that on creasted wheatgrass some races (such as L–3 and L–6) of *T. levis* and (T–2 and T–8) of *T. tritici* were several times as virulent as others (such as T–1, T–3, T–9, and T–11). The differences in the relative virulence of the races on slender wheatgrass are even greater. Race T–10 was especially virulent, being 10 to 14 times as much so on slender wheatgrass as were races L–7, T–6, and T–11. In comparing the virulence of *T. levis* and *T. tritici,* as species, on crested and slender wheatgrass selections, it is seen that *T. levis* is about 35 per cent the more virulent on crested wheatgrass, but is slightly less virulent on slender wheatgrass.

Much greater differences were discovered in the reactions of the 36 selections of crested and slender wheatgrass to the smut races than in the relative virulence of the latter. Although none of the 20 crested wheatgrass selections proved to be absolutely immune from *T. levis,* a few were highly resistant. Several others were moderately susceptible, with average percentages of infection of 24 to 32 per cent. One selection of crested wheatgrass appeared immune from *T. tritici,* some were highly resistant, and others moderately susceptible. *Agropyron pauciflorum,* as a species, showed more marked differences in varietal resistance and susceptibility to *T. levis* than did *A. cristatum.* Eight of the 16 selections tested appeared to be immune from all of the 8 races of *T. levis,* only 2 were moderately susceptible, and the remainder were more or less resistant. The same selections that were resistant to *T. levis* were, in general, also, resistant to *T. tritici.* One selection exhibited more or less susceptibility to each of the 11 races of *T. tritici.*

As a species, *Agropyron cristatum* is more susceptible to bunt of wheat than is *A. pauciflorum.*

Evidence is presented to show that the mycelium of *Tilletia levis* and *T. tritici* is perennial in perennial hosts, but not indefinitely, since in nursery rows the number of infected plants grows smaller each year. This is attributable to, (1) the death of some of the smut-infected plants, and, probably, (2) the death of the mycelium in other infected plants. Of 90 plants of *Agropyron cristatum, A. pauciflorum, A. subsecundum,* and *Hordeum nodosum* showing smut in 1935, 39 were in some manner freed of infection during the 2 years following, 7 died, and 44 still retained the disease in 1937. In 2 instances plants, apparently healthy in 1935, showed smut in 1936, but these showed no infection in 1937. In every case, plants that showed smut in 1935, but not in 1936, were still bunt-free in 1937. In general, it is probable that the duration of perennation of the mycelium of *T. levis* and *T. tritici* in their perennial hosts will be influenced by, (1) the degree of susceptibility of the host plant, (2) predisposition of the infected plants to drought or winter injury.

Smut balls of *Tilletia tritici* found in a sample of sweet clover seed, but obviously from some grass, were used to inoculate seed of crested wheatgrass, slender wheatgrass, and spring- and winter-wheat differentials. The results of 3 years of inoculations indicate that this collection is race T–8. This is the second incidence of the natural occurrence of *Tilletia tritici* on a forage grass.

It has been observed, throughout the course of the present investigations, that the bunted plants resulting from infection with *Tilletia tritici* tend to be more or less stunted.

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STUDIES IN THE NUTRITION OF FUNGI. II. EFFECT OF INOCULUM ON THE GROWTH OF THE COLONY[1]

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It is generally believed that the inoculum may exert a profound influence on the growth of the ensuing colony because nutrients and auxithals, present in the agar of the inoculum, may diffuse into the medium and induce growth responses. The writers know of no experimental data supporting such a supposition.[2] While in certain cases heavy or light "seeding" of spores of fungi and cells of bacteria may become important factors in the subsequent growth, here we are concerned only with the effect of the bit of agar removed from stock cultures and transferred to fresh media.

In this work thiamin (Vitamin B_1) and dextrose were used as test substances, and *Phycomyces blakesleeanus* as the test organism to demonstrate any possible influence of the inoculum upon the growth of the colony. *Ph. blakesleeanus* cannot grow without thiamin or its moieties, and is so sensitive as to show a discernible effect in the presence of 1 part of thiamin in 20 billion parts of medium (0.05 microgram per liter). The nutrient agar consisted of the following ingredients: 1 g. each of ammonium nitrate, dihydrogen potassium phosphate, and magnesium sulphate, a trace of Robbins' modification of Hoagland's A-Z mixture of rare elements, 5 g. of Bacto dextrose, 20 g. of Bacto agar, and 1,000 ml. of distilled water. The reaction of the medium was adjusted to pH 5.5. One part of thiamin in 100,000,000 parts of this medium induces a very good growth in *Ph. blakesleeanus*, while, without it, no growth results. The medium was divided into 4 lots and thiamin was added in the following proportions: 1 to 10,000, 1 to 100,000, 1 to 1,000,000, and 1 to 20,000,000. Fifteen ml. of each of the 4 lots of agar were poured in a series of plates (90 mm.) and inoculated with *Ph. blakesleeanus*. By means of a cork borer with an opening of 5 mm. in diameter, inoculum discs of uniform size and thickness were cut along the margin of a young, vigorously growing colony and transferred to plates of nutrient agar containing no thiamin. The cultures were incubated at 25° C. for 10 days. Inoculum-discs removed from the nutrient medium containing 1 part of thiamin in 20,000,000 failed to grow; those from agars containing 1 part of thiamin in 1,000,000 parts of the medium made a slight growth, while the inoculum-discs removed from plates containing the highest concentrations (1 part in 10,000 and 100,000) of thiamin gave rise to a fairly good colony. Since 1 part of thiamin in 100,000,000 parts of the medium induces excellent growth in *Ph. blakesleeanus*, in the stock cultures one must use 100 times the optimum amount before the inoculum begins to show even a slight effect, and from 1,000 to 10,000 times the optimum before a significant growth results.

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[2] After this work was submitted for publication we received a paper by Nils Fries (Über die Bedeutung von Wuchsstoffen für das Wachstum verschiedener Pilze, Symbolae Botanicae Upsalienses III: 2, 1–188, 1938) in which it is stated that inoculum pieces of 64–100 square millimeters induced much greater growth than inoculum pieces of 2.25 square millimeters.

A certain amount of diffusion of thiamin from the inoculum-discs of high thiamin content into the agar enabled the fungus to grow; but this growth was not as good as that made by control cultures (thiamin added to the agar at the rate of 1 part in 100,000,000). A Petri dish, 90 mm. in diameter, has a surface 320 times that of an inoculum-disc, 5 mm. in diameter. Such an inoculum-disc cut from a plate of agar containing 1 part of thiamin in 10,000 parts of the medium and transferred to 15 ml. of nutrient medium containing no thiamin would correspond to a thiamin concentration of 1:3,200,000, provided that all the original thiamin were still in the inoculum-disc and all of it managed to diffuse into the agar; however this is not the case, as can be seen from the results obtained in the following experiment: after a certain amount of diffusion from the inoculum-disc into the agar enabled the fungus to grow and fill the plate, the original inoculum pieces, which at the beginning of the experiment contained 1 part of thiamin in 100,000 and in 10,000 parts of the medium, were again removed from the colony and transferred to another series of plates of agar containing no thiamin. The inoculum-discs containing the smaller amount of thiamin (1:100,000) made slight growth, while those with the highest concentration (1:10,000) continued to induce fair growth and required a third transfer before losing their potency. Thus, it can be seen that in higher concentrations diffusion does not keep pace with the rapid growth of the fungus, and, once the colony has attained its full growth, it does not benefit from the surplus thiamin still present in the original inoculum.

The hyphae in the immediate vicinity of the inoculum seem to absorb and retain the diffused thiamin. In order to determine if this were true, the following experiment was made: 15 ml. of the nutrient medium containing no thiamin were poured in a number of 90 mm. plates (series A). In the second series (B) 15 ml. of nutrient medium containing an excess of thiamin (1 part of thiamin in 100,000 parts of the medium) were poured in a number of Petri dishes. By means of a cork borer with an opening of 5 mm. in diameter, discs were cut in the series B agar and were transferred to the plates in Series A. These discs were deposited on the surface of the agar at one edge of the plate, while the opposite edge was inoculated with inoculum-discs of *Phycomyces blakesleeanus*. Thus, the source of thiamin and the inoculum were separated by a distance of 80 mm. It should be stated here that, even in the abscence of thiamin, this fungus can form a few submerged hyphae that will reach throughout the agar in search of food. As soon as these submerged hyphae approached the outer limits of the thiamin diffused from the agar placed at the opposite edge of the medium, they began to grow vigorously and formed an abundant crop of aerial hyphae (Fig. 1). Eventually, one part of the plate was full of mycelium, while the other part, beginning at the inoculum, showed nothing but a thin, submerged growth that did not increase with time. Thus, the thiamin was cut off from the other part of the plate by the vigorously growing region of the colony. A coenocytic mycelium notwithstanding, the vitamin failed to be transported to the older parts of the colony that were starving for thiamin. This capacity of the vigorously growing mycelium to prevent diffusion throughout the medium is another reason why under ordinary conditions the food materials and auxithals present in the agar of the inoculum piece do not exert an appreciable influence upon the growth of the ensuing colony.

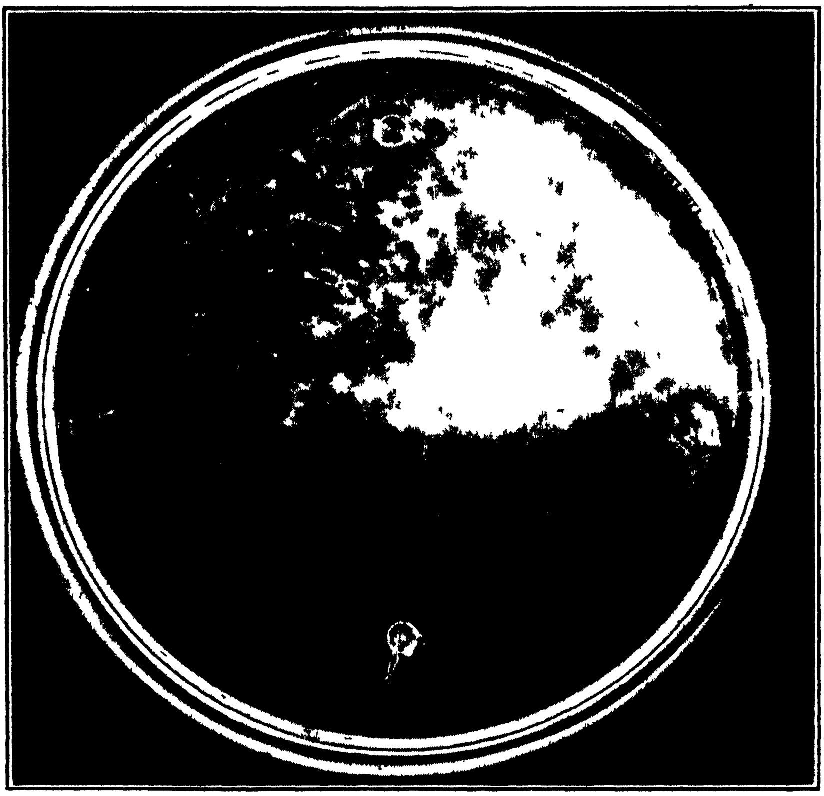

FIG. 1. Diffusion of thiamin into the agar. A, inoculum disc of *Phycomyces blakesleeanus;* B, a disc of agar containing 1 part of thiamin in 100,000 parts of the medium. A few scanty submerged hyphae (not the folds of agar itself showing as black lines) have advanced towards the source of thiamin and have formed a profuse growth in the area of diffused vitamin. No further growth occurred in the bare region extending from the inoculum to the zone of profuse aerial hyphae. (Shadowgraph by Dr. E. A. Marten.)

Only abnormally high concentrations of thiamin enable the inoculum to become a serious factor in the growth of the colony. There are very few substances that can be concentrated 2,000,000 times (from 20 billion to 10,000) without bringing about harmful effects upon a fungus; therefore, it is unlikely that nutrient substances needed for media will be used in abnormally high enough concentrations to be deciding factors in the growth of the new colony.

The foregoing and the following experiments were repeated several times but the results were always the same.

The Effect of Dextrose.—Experiments with various concentrations of dextrose gave uniformly negative results. Nutrient media were prepared with 1, 2, 5, and 10 per cent of dextrose, all with a thiamin content of 1 part in 20 million. Fifteen ml. of each of the agars were poured in plates and inoculated. Inoculum-discs cut from the margin of the colony were transferred to plates of agar containing the essential minerals and thiamin, but no dextrose or any other carbohydrate. No significant growth appeared in any of the cultures, despite the high concentrations of dextrose in the inoculum. This was to be expected because, even if all of the original amount of the dextrose (10 per cent) in the inoculum were to diffuse into the agar, it would yield not more than 1 part of dextrose in 3,200 parts of agar, too minute a quantity for growth. There was no increased growth, even in the immediate vicinity of the inoculum, where one would expect to find a concentration of the diffused dextrose. In all cases there was a slight submerged growth and very few, scattered aerial hyphae that were undoubtedly

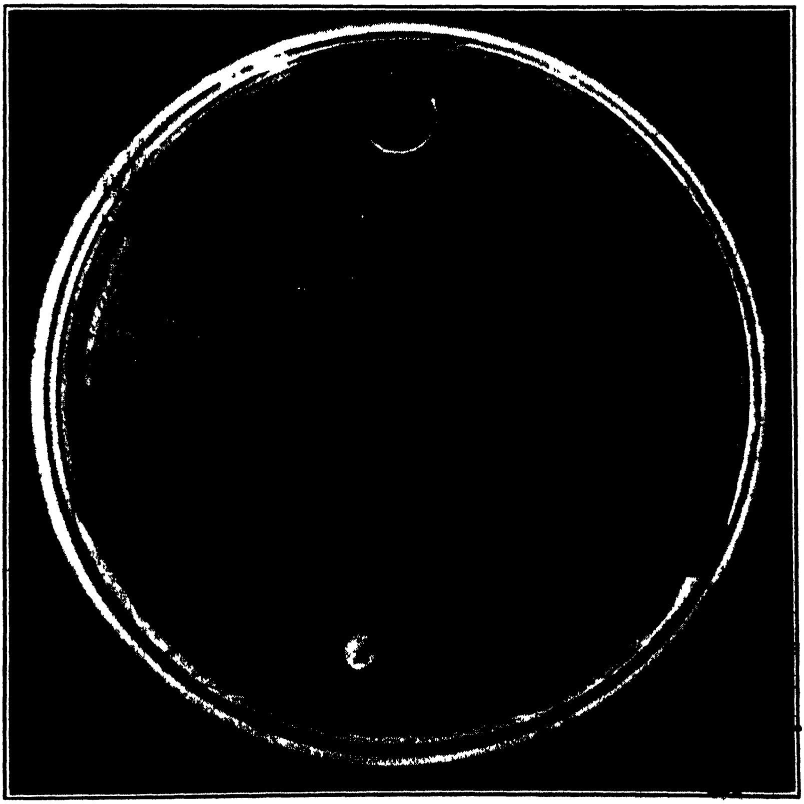

FIG. 2. Diffusion of dextrose through the agar. A, inoculum, B, disc of agar containing 20 per cent dextrose. The response is essentially the same as that in figure 1, except that the mycelium has not yet reached the source of dextrose.

induced by traces of carbohydrates or similar substances present in the agar-agar. The difference between the growth resulting from inoculum discs of the lowest and of the highest dextrose content was barely discernible.

The foregoing results should not convey the idea that dextrose does not diffuse readily through agar, but merely that there was not enough dextrose to supply the needs of the fungus. This was demonstrated by the following experiment: two series of agars were prepared, A and B, with 15 ml. of the medium in each plate. The A series contained the nutrient salts, thiamin and agar; the B series contained only 20 per cent dextrose and agar. Discs 5 and 10 mm. in diameter were cut from series B agar and transferred to a number of plates containing series A agar, each plate receiving a disc of dextrose agar at one edge and an inoculum disc of *Phycomyces blakesleeanus* at the opposite edge. The fungus began to make a scanty, submerged growth and advanced towards the source of dextrose. Where the discs cut from the dextrose medium were only 5 mm. in diameter, no appreciable increase in the growth resulted; but, in case of the 10 mm. discs, the submerged hyphae soon reached the diffused dextrose and made a rich growth, producing a colony one half of which was starving for dextrose, while the other half absorbed and retained all available sugar (Fig. 2).

The foregoing results suffice to show that for all practical purposes the quality and the quantity of diffusible substances present in the agar of the inoculum do not exert any material influence upon the growth of the ensuing colony.

SUMMARY

By using thiamin and dextrose as test substances, and *Phycomyces blakesleeanus* as test organism, it was demonstrated that the quality and the quantity of nutrients and of auxithals present in the agar of a given size inoculum are not of significant importance in the initiation and the development of the new colony.

ROOT-KNOT NEMATODE INJURY RESTRICTED BY A FUNGUS[1]

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(Accepted for publication February 20, 1939)

Among the natural enemies of the root-knot nematode, *Heterodera marioni* (Cornu) Goodey, thus far recognized in Hawaiian field and garden soils (5), the hyphomycetous fungi that capture larvae by means of highly specialized trapping mechanisms are perhaps the most widely prevalent. That such fungi occur widely elsewhere is evident from the work of Zopf (10), Sherbakoff (8), Couch (1), and especially Drechsler (2), but as yet they have received little consideration as possible aids in the control of plant-parasitic nematodes. To Drechsler, especially, goes credit not only for the discovery and taxonomic treatment of many such species but also for the elucidation of their predaceous mechanisms. Through observations in Petri dishes of agar, he and others have demonstrated the capacity of such fungi to destroy nematodes in large numbers.

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[1] Published with the approval of the Director as Technical Paper No. 119 of the Pineapple Experiment Station, University of Hawaii.

Evidence as to how these fungi act in soil is meager. In pure culture, they grow readily as saprophytes on various media, but it is Drechsler's opinion (2, p. 448), which our observations support, that, in nature, they probably obtain most or all of their food from nematodes. There is no doubt that they capture and destroy larvae of *Heterodera marioni* and other nematodes in soil, for we have observed the trapping mechanisms and mycelium of several species attached to living and dead nematodes freshly washed from soil samples. As a group, they appear to be important members of the biological control complex which, we have demonstrated, reduces *H. marioni* populations decisively during decomposition of organic matter in soil (6). We find them abundantly associated with nematode-infested roots and isolate them readily by placing pieces of such roots, washed thoroughly and drained, onto the surface of water agar. Heretofore, however, there has been no experimental evidence that these fungi are able to limit the rise of an *H. marioni* population around the roots of a susceptible plant sufficiently to restrict the severity of nematode injury. An experiment that provides such evidence for one fungus, *Dactylella ellipsospora,* is reported here.[2]

PLAN OF THE EXPERIMENT

This experiment involved the growth of pineapple plants, *Ananas comosus* (L.) Merr., in sterilized soil infested with larvae of *Heterodera marioni,* both with and without the addition of cultures of 5 species of nematode-trapping fungi. There were 10 experimental treatments (Table 1), each consisting of

TABLE 1.—*Key to experimental treatments*

Treatment symbol	Additions	
A	Desiccation-tolerant microorganisms.	
B	" " " .	Sterile bagasse medium.[b]
C	Nematodes,[a] 490 per pot.	
D	" , " " " .	Sterile bagasse medium.
E	" , " " " .	*Arthrobotrys oligospora.*[c]
F	" , " " " .	*Dactylella ellipsospora.*
G	" , " " " .	*Arthrobotrys musiformis.*
H	" , " " " .	*Dactylaria candida.*
I	" , " " " .	*Dactylaria thaumasia.*
J	" , " " " .	*Arthrobotrys oligospora.*

[a] Added in suspension, with contaminating microorganisms including other species of nematodes.

[b] Sugar-cane bagasse, wet with tap water and sterilized, added at the rate of 25 grams per pot of soil.

[c] This fungus and those following were added in culture on bagasse at the rate of 25 grams per pot.

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[2] This has already been reported in abstract (4).

10 five-gallon pots with one plant per pot. Uniform soil with a small admixture of peat moss was sterilized in the pot with steam under pressure. After it had cooled, cultures of nematode-trapping fungi, grown in sterilized sugarcane bagasse moistened with tap water, were then stirred into the upper 6 inches of soil for 6 of the treatments—approximately 25 grams of culture per pot. To provide adequate checks, there was mixed into pots of 2 treatments a like quantity of the sterile bagasse medium, without the trapping fungi. Slips of one clone of the Cayenne variety, matched for size and treated with hot water to minimize chance infestations, were then planted.

In the absence of pure cultures of *Heterodera marioni*, larvae to infest treatments C to J were hatched from egg masses on cowpea roots grown in naturally infested soil. The roots, after thorough washing, were kept wet overnight and, the following morning, the freshly hatched larvae were concentrated with as little debris as possible in 1100 cc. water. While this suspension was agitated to prevent settling, three 10 cc. aliquots were withdrawn for nematode counts. Then, 10 cc. quantities of this suspension were added to the soil at the side of the pineapple slip in each pot of treatments C to J. The cowpea roots were kept wet an additional 24 hours, then this process was repeated. Counts of nematodes per 10 cc. were 171, 141, 163 the first day, and 350, 312, 334 the second day, indicating that the mean number of larvae added per pot approximated 490. Unavoidably, this suspension carried a mixture of diverse microorganisms. To introduce at least some of these into treatments A and B, without the root-knot nematode, 200 cc. of each day's suspension were poured onto quartz sand and reduced to the air-dry condition to kill *H. marioni;* then, this sand mixture was divided by weight into 20 equal parts and added to the 20 pots of these two treatments. This sand carried, in viable condition, numerous saprophytic fungi in addition to various bacteria and protozoa, as was determined by plating small quantities of the sand on various agar media. Thus, it provided the nematode-free checks with complex soil flora and fauna somewhat comparable to the nematode-infested treatments.

The experiment provided 2 comparisons of uninfested checks, A and B, with 2 nematode-infested checks, C and D, to measure the severity of nematode injury, both with and without bagasse. Then, for comparison with the nematode-infested checks, there were included 6 other infested treatments with cultures of trapping fungi added as follows: 2 (E and J) with *Arthrobotrys oligospora* Fres. from two separate isolations, and one each with (F) *Dactylella ellipsospora* Grove, (G) *Arthrobotrys musiformis* Drechsler, (H) *Dactylaria candida* (Nees) Sacc., and (I) *Dactylaria thaumasia* Drechsler. For details concerning these fungi, 2 of which trap with adhesive knobs and the others with three-dimensional nets, the reader is referred to Drechsler (2).

This experiment, of Latin square design, was arranged in Latin square formation on tables out-of-doors to equalize effects of exposure to wind and sunlight. The 10 rows and 10 columns of 10 plants each had superimposed on them 2 secondary variables. It was impossible to prepare the necessary quantity of soil in a single batch: it was prepared as similarly as possible in 5 batches and these were distributed randomly in rows. Similarly, although the vegetative slips planted were relatively similar in size, it was impossible to obtain, from one clone, 100 of precisely the same weight; consequently, slips were matched closely within 10 slip-size classes[3] and these classes were distributed randomly in columns. This arrangement made it impossible to distinguish between variance due to soil batch, slip size, and exposure, but it was desirable because it favored analysis for differences between treatments, permitting the elimination of variance due to these other factors from the residual experimental error.

Quantitative data to measure plant growth and nematode injury were taken after approximately 15 months, during which the experiment received uniform fertilizer applications, insect control treatments, etc. In analyzing the root data by the method of variance (9), it appeared advisable to omit treatments A and B, the nematode-free checks, since these fell too far apart from the remainder of the experiment, but Latin square analysis was still made on the basis of 10 rows and 10 columns of now only 8 plants each, with degrees of freedom properly adjusted.

ESTABLISHMENT AND PERSISTENCE OF THE FUNGI

To determine what nematode-trapping fungi were still active after 15 months, selected pieces of root bearing old galls were washed thoroughly, drained and transferred to Petri dishes of water agar. These dishes, incubated 2 or 3 weeks at room temperature, were examined thoroughly at least twice. There was only one Petri dish per plant, with 4 to 7 pieces of root, and experience shows this to be too small a sample to detect all predaceous fungi present. It does serve, however, to reveal the most abundant or most active fungus associated with a root system. In this way, 8 to 10 root systems from each nematode-infested treatment were sampled, with results as summarized in table 2. Results are expressed as percentages because of unequal numbers of plants sampled per treatment.

Among the 72 plants sampled, all but 5 yielded cultures of at least one species of trapping fungus, and 3 of these 5 were plants of treatments C and D to which no cultures had been added. Within these 2 treatments, 89 per cent and 75 per cent, respectively, of the plants yielded cultures of one or more such fungi, indicating a very high chance infestation. *Dactylella ellipsospora* and *Arthrobotrys oligospora,* the 2 dominant fungi here, were also dominant in the field source of the nematode infestation.

Among the 55 plants sampled from treatments E to J, experimentally infested with fungi at the outset, the same fungus added was reisolated from 51 plants or 93 per cent. From 2 of the remaining 4 plants, trapping fungi other than those added were recovered. Failure of reisolation occurred only

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[3] Individual slips ranged in weight from 92 grams to 140 grams but means of slip-size classes ranged from only 95.3 grams to 137.4 grams. With each treatment including one slip of each size class, the treatment means of slip weight ranged only from 113.0 grams to 116.1 grams.

TABLE 2.—*Results of sampling root systems for nematode-trapping fungi after 15 months growth*

Treatment	Plants sampled no.	Fungus added	Plants from which fungi were recovered, per cent						Other natural enemies[a]
			Arthrobotrys oligospora	*Dactylella ellipsospora*	*Arthrobotrys musiformis*	*Dactylaria candida*	*Dactylaria thaumasia*	None	
C	9	None	22	67	0	0	11	**11**	0
D	8	None	37	62	0	0	0	**25**	0
E	9	*A. oligospora*	**78**	33	0	11	0	11	*Dorylaimus* sp.,[b] 1 plant.
F	9	*D. ellipsospora*	11	**100**	0	0	0	0	*Dorylaimus* sp.,[b] 1 plant.
G	10	*A. musiformis*	0	10	**90**	20	0	10	*Dorylaimus* sp.,[b] and *Catenaria anguillulae*,[c] 1 plant each.
H	9	*D. candida*	0	33	0	**100**	0	0	0
I	9	*D. thaumasia*	0	33	0	0	**100**	0	*Harposporium anguillulae*,[d] 1 plant.
J	9	*A. oligospora*	**89**	44	0	0	0	0	*Harposporium anguillulae*[d] and *Dactylella bembicodes*,[e] 1 plant each.

[a] The egg parasite, *Penicillium* sp., was present throughout the experiment on exposed and shallowly embedded egg masses.

[b] Two species of *Dorylaimus*, both aggressively predacious on smaller nematodes.

[c] A chitridiaceous fungus parasitic on nematodes.

[d] A fungus parasitic on certain nematodes but apparently not on *H. marioni*.

[e] A nematode-trapping fungus not used in any prior experiment in this vicinity.

in treatments E and J, infested with 2 isolates of *Arthrobotrys oligospora* and in treatment G, infested with *A. musiformis*, the other 4 species being reisolated from every plant experimentally infested with them.

Contaminating species of nematode-trapping fungi were present even where pure cultures had been added. With only this inadequate sampling, 49 plants yielded a single species, 17 yielded 2 species and one plant yielded 3 species. In addition, as recorded in the footnotes of table 2, there were various other enemies of nematodes present as chance contaminants. *Dactylella ellipsospora,* the dominant contaminant in the noninfested treatments C and D, was reisolated from at least one plant from every treatment. At the opposite extreme, *Arthrobotrys musiformis* was isolated only from plants to which it had been added experimentally, and *Dactylaria thaumasia* was not isolated from any plants infested with another species. The data of table 2 demonstrate that it is possible to modify a chance mixture of nematode-trapping fungi by the addition of a culture, for it is plain that a fungus added experimentally 15 months earlier had dominated other species present by chance and maintained this dominance. Both *D. ellipsospora* and *A. oligospora,* though present as contaminants, were reisolated more frequently from plants infested experimentally with these fungi than from the naturally infested treatments C and D, and were reisolated less frequently when some other fungus was added in pure culture.

The most probable source of the chance infestation was the nematode suspension added at the outset, for it has been our experience that large numbers of *Heterodera marioni* larvae obtained as these were always carry natural enemies. There may also have been some contamination from wind-blown conidia, since these fungi are known to sporulate on the surface of sterilized soil. Still, *Arthrobotrys musiformis,* not once found as a contaminant, sporulates readily under these conditions. The egg parasite, *Penicillium* sp., which was present throughout the experiment, was abundant on the cowpea roots from which the *H. marioni* larvae were obtained, and the predaceous nematodes of the genus *Dorylaimus,* as well as the chitridiaceous fungus, *Catenaria anguillulae,* most probably were introduced with the nematode suspension.

The chance establishment of natural enemies of *Heterodera marioni,* and especially the relative abundance of *Dactylella ellipsospora* in the checks, C and D, is emphasized because this prevented our obtaining a measure of the full benefits accruing from the action of trapping fungi in restriction of root-knot injury. Treatments with cultures added must be compared with heavily infested checks rather than with trapper-free checks. Despite this, evidence was obtained that at least one fungus, *D. ellipsospora,* does restrict root-knot injury, but the extent of this restriction cannot be estimated.

NEMATODE POPULATIONS IN SOIL

Immediately before plants were removed for examination, composite soil samples were taken for nematode counts from near the surface in 4 pots of each treatment. Root fragments with adhering soil were eliminated to avoid the disturbing influence of nematode conncentrations around roots. A 50-gram sample of soil from each pot was soaked in water, then the nematodes were separated by the usual technique of decanting and wet screening, examined microscopically, and counted. Nematode populations so obtained were low, averaging only 193 per 100 grams of soil for the experiment as a whole. Of these, 89 per cent were species of *Cephalobus,* 7 per cent were *Pratylenchus pratensis,* and only 4 per cent were larvae of *Heterodera marioni.* These other nematodes, introduced in small numbers with the original infestation of *H. marioni* larvae, had thrived under the experimental conditions, but it must not be assumed that the relative numbers recorded here were representative of the entire pot, because large numbers of *H. marioni* were present within the roots but the *Cephalobus* spp., microphagous and saprophagous in habit, were chiefly free in the soil.

As is to be expected with these small populations, the counts from separate pots varied widely and the significance of differences between treatments is left in doubt. Treatment F, however, infested with *Dactylella ellipsospora,* was the only treatment from which no live *Heterodera marioni* larvae were obtained and also was the treatment with the lowest total population of nematodes of all kinds.

MEASUREMENT OF NEMATODE INJURY

Plants were removed from the soil, one slip-size class at a time, dead leaves were removed from the stem base, the roots were washed, then each root system was photographed at a uniform scale. A pineapple root system, somewhat comparable to that of corn after its primary root has been lost, consists entirely of adventitious roots and laterals developed from them. Data on root number and root length included only the adventitious roots themselves, ignoring laterals. Root weight, however, included the entire root system and was determined after uniform blotting and draining.

Root systems of treatments A and B, the nematode-free checks, were much more extensive than those usually found on vigorous field-grown plants of corresponding age. The oldest roots were still alive and new roots had continued to develop throughout the entire period of growth. A very few galls were found on some plants, apparently resulting from late chance infestation.

Root systems of plants of the 8 nematode-infested treatments were comparatively meager, were heavily galled and, in addition, exhibited some lesions of *Pratylenchus pratensis*, the meadow nematode. Old galls and some of the older roots were necrotic but there was no evidence of any aggressive root-rotting fungus. The earliest formed roots bore relatively few galls, with these few chiefly on small laterals. Godfrey (3) has shown that a large concentration of *Heterodera marioni* is required to form a terminal gall on an adventitious root of pineapple, and the initial infestation of approximately 490 larvae per 5-gallon pot of soil was insufficient to provide such concentration. Later roots, however, developed after multiplication of nematodes, exhibited numerous nonterminal and some terminal galls with still more galls on lateral roots, and most of the young roots, developed during the last few months' growth, had been stopped short by large terminal galls. Such roots had developed few short laterals, most of which were galled.

Among these 8 treatments, only F appeared consistently superior to the others. Here, the bulk of root systems was visibly greater, not only from a greater number of adventitious roots but also from a greater length of these and a greater profusion of laterals. Most of the more recent roots bore at least a few galls, but the nematode population had been insufficient to stop the growth of all of them. Figure 1 shows that roots of this treatment, infested with *Dactylella ellipsospora*, were on the average distinctly superior to the appropriate check, treatment D, but were still far inferior to the nematode-free check, treatment B.

The quantitative data on root and top condition, summarized in table 3, show both nematode-free checks, A and B, to be consistently and markedly superior to all other treatments. In agreement with figure 1, the data demonstrate treatment F, infested with *Dactylella ellipsospora*, to be superior to the other nematode-infested treatments in several plant growth characters.

TABLE 3.—*Mean top and root data from pineapple plants grown 15 months from slips*

Characters compared		Without bagasse		With bagasse							
		Check	Nema-tode check	Check	Nema-tode check	*Arthrobotrys oligospora*	*Dactylella ellipsospora*	*Arthrobotrys musiformis*	*Dactylaria candida*	*Dactylaria thaumasia*	*Arthrobotrys oligospora*
		A	C	B	D	E	F	G	H	I	J
Top weight increase	%	1369	828	1384	914	940	994	863	888	928	878
Greatest leaf length	cm.	79	63	77	65	66	71	66	67	66	66
Fresh weight of roots	g.	319	52	299	53	63	**102**	60	61	58	55
Total root length	cm.	4300	1176	4120	1278	1356	**1783**	1272	1368	1263	1352
Gall-free length of roots	cm.	4299	1106	4109	1177	1279	**1685**	1205	1298	1178	1248
Length gall-free roots	cm.	4282	537	4017	555	623	**877**	498	567	588	560
Number of roots	no.	222	114	221	122	129	**149**	111	120	116	125
Gall-free roots	%	99	56	96	57	60	59	52	52	57	53
Main-root galls	no.	1	51	7	59	50	58	46	51	48	64
Lateral-root galls	no.	2	366	7	392	454	443	470	480	461	357
Total galls	no.	3	417	14	451	504	501	516	531	509	420

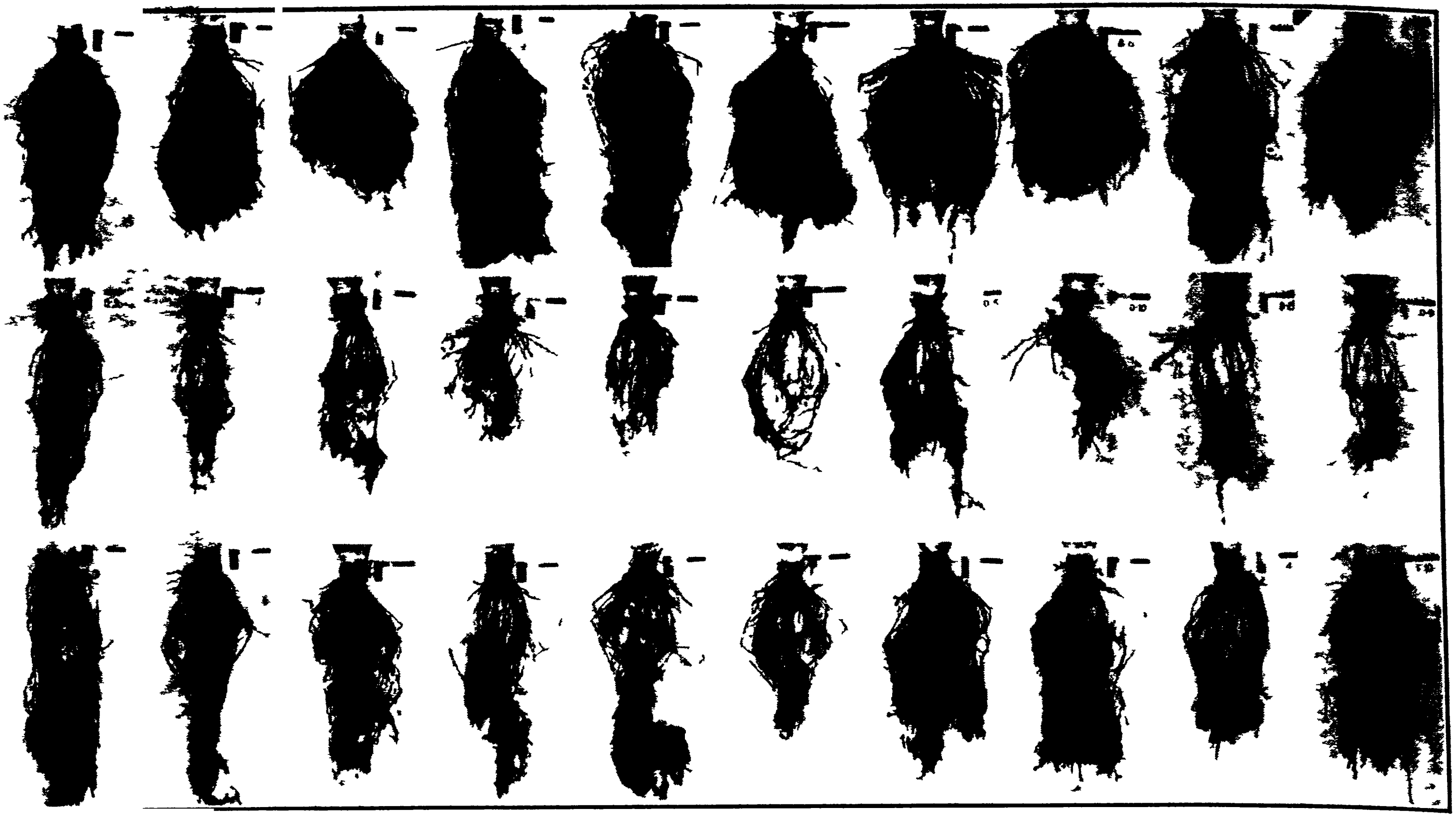

FIG Photographs o all root systems of three treatments above, treatment B, bagasse but no nematodes, middle, treatment D, bagasse .h *H* *oni*, below, tre ment F, *H marioni* larvae plus bagasse culture of *Dactylella ellipsospora* Scale uniform throughout

Mean values for this treatment that were found by analysis of variance to be statistically superior to the more valid check D, are indicated by bold-face type.

To facilitate comparison, percentage differences from appropriate checks have been recorded in table 4. First, to measure the effects of nematodes with and without bagasse and to indicate the sensitivity of various plant characters to nematode injury, treatment A is compared with C and treatment B with D. Slightly, but not significantly, less injury was recorded where bagasse was added, presumably as a result of the decomposition effect reported elsewhere (6). Total length of gall-free roots proved to be the most sensitive indicator of nematode injury, with nematodes causing approximately 87 per cent reduction from the nematode-free checks. Fresh weight of root systems was the second most sensitive indicator, with a reduction of 83 per cent, while the third most sensitive was gall-free length of roots, a measure of the sum of lengths of adventitious roots from the stem base out to the first gall on the main root axis. Criteria of top growth proved much less sensitive, with reductions, attributable to nematodes, of 39 per cent without bagasse and 34 per cent with bagasse. Under these conditions of culture, the nematode-free check plants appear to have had more extensive root systems than they required. Statistical significance of these differences between nematode-infested treatments and the two nematode-free checks was very high throughout.

TABLE 4.—*Effects of the root-knot nematodes on pineapple top and root growth during 15 months, with and without bagasse and a culture of Dactylella ellipsospora*

Characters compared	Reduction from check		Increase from nematode-check		
	Nematodes alone (A–C, % of A)	Nematodes plus bagasse (B–D, % of B)	Fungus plus bagasse (F–C, % of C)	Fungus itself (F–D, % of D)	Fungus itself Odds[a]
Top weight increase, %	39.5	34.0	20.0	8.8	9: 1
Greatest leaf length	20.2	15.6	12.7	9.2	390: 1
Fresh weight of roots	83.7	82.3	96.2	92.5	3690: 1
Total root length	72.6	69.0	51.6	39.5	540: 1
Gall-free length of roots	74.3	71.3	52.3	43.2	620: 1
Length of gall-free roots	87.5	86.2	63.3	58.0	480: 1
Number of roots	48.6	44.8	30.7	22.1	38: 1
Gall-free roots, %	43.9	41.2	5.0	3.3	2: 1
Main-root galls	......	......	13.7	-1.7	1: 1
Lateral-root galls	......	......	21.0	13.0	2: 1
Total galls	......	......	20.1	11.1	2: 1

[a] From Miles' table (7) and the standard error of the experiment as a whole determined by the method of variance.

Table 4 also compares treatment F with treatments C and D. The comparison with C provides a measure of the aggregate action of bagasse and *Dactylella ellipsospora*. The more valid comparison, however, is between F and D, since both of these received additions of nematodes and bagasse. Differences in this comparison are somewhat less than in the former one. These differences have been calculated as percentage increases from the nematode-infested checks to provide a measure of improvements brought about by this fungus. Finally, to indicate statistical significance of the more valid comparison, this table includes a column of odds.

In leaf length, fresh weight of roots, total root length, gall-free length of roots, length of roots free from galls, and total number of roots, the addition of the *Dactylella ellipsospora* culture gave statistically significant improvement. The 9 per cent increase in top weight lacks significance because of excessive variability but, from the magnitude of other differences, it is probable that during a longer period of growth, including fruit development, significant differences in this character would have developed. Numbers of galls per root system were not reduced by the action of this fungus but, on the contrary, were increased slightly. This increase, however, was not nearly proportional to the increase in extent of root system. Very severe injury results in such meager root systems that large numbers of galls are not to be expected.

Relative rankings of treatments infested with other species of nematode-trapping fungi are indicated sufficiently by the following figures on fresh weight of root systems. Treatment F showed a 92.5 per cent improvement over the appropriate check D. The similar comparison for other fungi is as follows: *Arthrobotrys oligospora,* 2 isolates, 18.9 per cent and 3.8 per cent, respectively; *A. musiformis,* 15.2 per cent; *D. candida,* 15.1 per cent; *D. thaumasia,* 9.4 per cent. Though not significant by themselves, these differences were paralleled by slightly smaller differences in the same direction in several other characteristics and suggest that some of these other species of fungi had reduced nematode injury very slightly.

DISCUSSION

The evidence here presented that a nematode-trapping fungus is capable of so restraining the rise of a population of *Heterodera marioni* during the growth of a susceptible plant as to restrict the severity of nematode injury, indicates that such fungi are beneficial constituents of the soil flora and suggests a possible approach to the control of plant-parasitic nematodes. Although this experiment proves the value of only one fungus, *Dactylella ellipsospora,* the results indicate strongly that other fungi act similarly but to a lesser degree. With the heavy chance infestation of this fungus in the nematode-infested checks, it is rather surprising that the increased infestation obtained by adding a culture of this same fungus was able to restrict nematode injury to a degree permitting statistical demonstration.

The superiority of *Dactylella ellipsospora* found here had not been anticipated on the basis of observations in agar, since, under those conditions, *Arthrobotrys oligospora* and *Dactylaria thaumasia,* which capture nematodes in three-dimensional nets, have commonly appeared more aggressively predaceous than this fungus with its adhesive knobs. There is no reason to suppose that *Dactylella ellipsospora* will prove consistently superior to the other species in experiments conducted under different conditions, for we do not yet know how nematode destruction by these fungi is influenced by such environmental variables as soil type, composition, reaction, moisture, and temperature. It is evident, however, that comparative tests during plant growth in soil will be required to determine relative efficiencies of biological agents in nematode control.

The full degree of effectiveness of predaceous fungi is still to be measured since this experiment failed to provide an infestation of *Heterodera marioni* free from its natural enemies, but, plainly, that degree will fall far short of eradication. These fungi apparently differ as to the density of a nematode population that they will permit to build up around the roots of a susceptible plant, and even the more effective fungi may give different results, depending upon the thoroughness with which they infest the soil.

The degree of nematode control of which such fungi are capable may vary with the host plant of the nematode and may also be of different value for some plants than for others. In pineapple roots, it is unusual for a single gall to be infested by larvae of succeeding generations. In the roots of some dicotyledonous plants, however, such repeated infestations commonly occur, with the result that the larval progeny of a single nematode may multiply within a large gall where predaceous fungi cannot reach them. Moreover, the commonly observed variability in tolerance of the root-knot nematode among plants more or less susceptible, suggests that some highly susceptible plants would suffer severe nematode injury despite the protection afforded by trapping fungi, while more tolerant plants would show striking benefits. It appears, accordingly, that the value of nematode-trapping fungi must be evaluated not only for distinct species of fungi under varying environmental conditions but also must be tested in association with different host plants.

The present meager evidence is insufficient to evaluate the probable future place of predaceous fungi in control of plant-parasitic nematodes but it is suggestive in several directions. Where such fungi already occur abundantly in association with nematodes, they are already helping to limit the density of nematode populations. The potential rate of multiplication of *Heterodera marioni* under favorable conditions is so great, however, that severe injury to host plants may result, even where large percentages of the larvae of every generation are destroyed by their enemies. Where nematode injury continues in the presence of a mixture of such fungi, the possibility is suggested by this study that a more effective fungus may be introduced in pure culture and that it may survive, dominate the less efficient fungi already present. and give an increased degree of nematode control. Where such fungi are not already established the likelihood of benefits from introduction of cultures seems much greater. It is desirable, therefore, that the testing of nematode-trapping fungi be expanded to include all species available and that it involve not only such pot culture tests as reported here but also experimental testing under field conditions in association with varied host plants of the nematodes. The present results give only a suggestion as to what may be learned in such tests where contrasting and fluctuating inanimate environments are encountered and where the predaceous fungi are in competition with the balanced flora and fauna of natural soils. Even the present evidence, however, establishes the fact that nematode-capturing fungi are beneficial members of the soil flora that should be considered in the application of direct measures of nematode control and in the shaping of agricultural practices generally, in order to permit them to operate at their maximum efficiency in the destruction of plant-parasitic nematodes.

SUMMARY

The relative severity of *Heterodera marioni* injury to pineapple was measured in a pot culture experiment in which sterilized soil was infested with larvae of this nematode, with and without additions of pure cultures of five species of nematode-trapping fungi. During 15 months of continuous plant growth, *Dactylella ellipsospora* restricted nematode injury to a moderate but statistically significant degree. A heavy chance infestation of trapping fungi and a lighter infestation of other enemies of nematodes, occurring where no cultures were added, prevented the measurement of the degree of nematode control achieved by this and other fungi. Each species, when added in pure culture, dominated the mixture of fungi occurring as contaminants and was reisolated in a high percentage of cases after a period of 15 months.

The fungus dominant among the contaminants was the species found most efficient when added experimentally. Consequently, the failure of other species to give statistically significant improvement in plant growth merely indicates that, when they were dominant, they were less efficient than the chance mixture of contaminants in which *Dactylella ellipsospora* was dominant.

Species of predaceous fungi that capture and destroy nematodes readily in agar differ in their ability to restrict nematode injury to susceptible plants in soil. Soil tests, therefore, appear necessary to evaluate efficiencies of such fungi.

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METHODS OF DETERMINATION OF PHYSIOLOGIC RACES OF RHIZOCTONIA SOLANI ON THE BASIS OF PARASITISM ON SEVERAL CROP PLANTS[1]

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INTRODUCTION

Physiologic specialization in *Rhizoctonia solani* Kühn has been demonstrated by a number of investigators. Before the prevalence and importance of physiologic races can be fully determined, it is necessary to establish criteria and evaluate methods for the differentiation of isolates into definite categories.

These investigations were made to study the variability in pathogenicity of a number of isolates in relation to possible identification of physiologic races of the organism.

METHODS AND MATERIALS

Damping-off experiments were made in 1931, 1934, and 1935, in the greenhouse, with 29 isolates of *Rhizoctonia solani* obtained from potatoes and sugar beets, principally from Minnesota. The following 3 crop plants were tested as possible differential hosts: sugar beets,[2] Detroit Dark Red table beets, and Grimm alfalfa. The procedure followed was similar to that used in damping-off experiments in previous studies (3).

In more extensive experiments, direct inoculations were made of underground stems of older plants of a number of crop plants. Inoculations were made by placing a small quantity of grain inoculum[3] of the particular isolate against the stem about ½ in. below the surface of the soil. The controls received similar treatment, except that only steamed grains were used. In most cases the inoculations were repeated at several different intervals. Because of limitations of greenhouse space it was necessary to test only 13 isolates, obtained from potatoes and sugar beets from widely different localities.

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[1] The data presented in this paper were obtained in cooperative investigations by the Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station. Paper No. 1674 of the Journal Series of the Minnesota Experiment Station.

[2] K. W. Normal brand, produced by Zuckerfabrik Kleinwanzleben.

[3] For sake of brevity, cultures grown on steamed oat and wheat kernels are referred to as "grain inoculum."

EXPERIMENTAL DATA

Damping-off Tests

The average percentages and coefficients of variability of damping-off of seedlings of sugar beets, table beets, and alfalfa caused by 29 isolates of *Rhizoctonia solani* are given in table 1.

TABLE 1.—*Comparative virulence of 18 potato and 11 sugar-beet isolates of Rhizoctonia solani as determined by the amount of damping-off of seedlings of sugar beets, table beets, and alfalfa in 3 experiments, each consisting of 5 replications*

Accession number[a]	Isolate	Hosts and percentage of damping-off					
		Sugar beet		Table beet		Alfalfa	
	Source	Damping-off	Coeff. of variability	Damping-off	Coeff. of variability	Damping-off	Coeff. of variability
P-20	Dilworth, Minn.	7.8	93.6	37.9	23.2	24.1	1.4
P- 7	Grand Forks, N. D.	8.8	62.0	11.5	42.4	27.4	21.5
P-16	Dilworth, Minn.	16.0	60.0	21.5	48.1	25.5	44.8
P-21	Grand Forks, N. D.	16.1	92.4	18.9	55.6	43.1	39.0
P-19	Moorhead, Minn.	16.8	50.3	21.7	19.7	29.1	33.8
P-15	Dilworth, Minn.	20.2	41.5	18.8	1.5	24.1	13.7
P- 3	Guthrie, Minn.	20.4	12.6	32.8	64.4	21.0	22.5
P-10	Glyndon, Minn.	25.0	54.8	36.8	42.0	47.5	9.6
P-12	St. Agathe, Canada	25.2	16.3	40.7	52.6	33.0	26.3
P- 9	Glyndon, Minn.	26.5	51.4	32.7	61.2	28.8	34.3
P- 1	St. Paul, Minn.	30.1	13.7	43.4	52.2	28.9	6.7
P-14	Dilworth, Minn.	30.4	46.9	31.0	60.8	26.2	81.5
P-13	Ste. Agathe, Canada	30.6	39.5	39.3	15.4	58.2	20.9
P-18	Moorhead, Minn.	30.8	33.4	35.0	59.7	27.6	35.9
P-11	Glyndon, Minn.	32.3	35.3	39.8	50.0	31.3	32.5
P-22	Dilworth, Minn.	35.2	28.5	56.4	36.1	21.8	46.6
P-23	Dilworth, Minn.	40.8	48.1	40 5	48.5	44.9	30.3
P- 4	Fosston, Minn.	47.4	20.5	33.9	60.3	22.4	16.0
SB-37	Michigan	78.3	15.3	63.5	30.2	82.6	5.2
SB-18	Mankato, Minn.	81.6	22.5	80.0	25.0	43.1	47.0
SB-33	Oslo, Minn.	82.2	21.6	90.1	10.9	50.5	39.8
SB-45	California	83.6	15.4	73.3	21.3	95.7	2.2
SB-23	Mankato, Minn.	85.2	17.4	77.3	29.4	57.7	37.0
SB-28	Mankato, Minn.	87.9	13.8	92.7	8.8	54.1	43.6
SB-43	Ohio	89.3	6.6	85.7	11.6	36.9	23.0
SB-49	Michigan	89.4	5.9	83.7	7.3	93.4	5.8
SB-39	Michigan	97.2	2.4	90.8	4.1	99.6	0.4
SB-50	East Grand Forks, Minn.	97.4	1.5	92.8	4.5	81.2	11.3
SB-13	Chaska, Minn.	98.4	1.3	93.1	0.8	61.4	35.0

[a] P = Potato isolate; SB = Sugar-beet isolate.

From the data it appears that the amount of damping-off that occurred in the 3 different experiments was very variable for most of the isolates tested, as indicated by the large values of most of the coefficients of variability. On the other hand, the degree of variability for a few isolates, for example, SB-39 and SB-49, was relatively small for each of the 3 hosts.

This divergence in amount of damping-off in successive tests probably is attributable to variations in soil environmental conditions, which might be explained in part, at least, by the fact that the experiments were made in different years and not always during the same period of the year. It would seem, therefore, that unless such factors as soil moisture, soil temperature, and soil texture can be more completely controlled, any differentiation of isolates into definite physiologic races on the basis of damping off of crop hosts is not advisable.

DIRECT-INOCULATION TESTS

Since the virulence of a great many isolates varied so greatly in the amount of damping-off of certain hosts, it was thought advisable to test the pathogenicity of a number of isolates by direct inoculation of the underground stems of the following crop plants: beans (6 varieties), pea, celery, tomato, cabbage, and carrot.

Beans

The 6 varieties of beans tested were Currie's Rust Proof, Bountiful, Pencil Pod Black Wax, Kentucky Wonder, Giant Stringless, and Manchu soybeans. The first 5 varieties are all garden string beans.

Three types of infection, produced by *Rhizoctonia solani* on the underground stem, were recognized in determining the relative virulence of the isolates to the different varieties of beans, as follows:

Type 0 = infection either not occurring or inducing only slight discoloration at point of inoculation.

Type 1 = lesions small to medium, shallow.

Type 2 = lesions medium to large, deep, leading to extreme decay or death of plant.

In the determination of host reaction to a given isolate, each plant was examined and classified as to infection type. Plants of the same infection type were grouped and the total number in each group was recorded. From these data the average infection type for each host was calculated for each isolate, which is the ratio of the total sum of products of each infection type by the number of plants contained therein to the total number of plants inoculated. This average infection type was then converted to a percentage basis, which is taken as the index of relative susceptibility.

Thus, the relative susceptibility index for soybeans to isolate SB-43 in inoculations made July 17, 1934, was 59.0 per cent. In this case 1 plant was type 0, 25 were type 1, and 7 were type 2. The average infection type was computed in the following manner.

$$\frac{(1\times 0=0)+(25\times 1=25)+(7\times 2=14)}{33}=\frac{39}{33}=1.18$$

This value (1.18) was then divided by 2 and multiplied by 100 to convert to percentage. Similar infection ratings were determined for each isolate on each host for each independent set of inoculations.

The relative susceptibility of 6 varieties of beans, which are the results of inoculating approximately 9000 plants, is given in table 2. Less variability in results between successive experiments occurred with this method of inoculation than in the damping-off experiments.

However, the degree of variability by direct inoculation of varieties of beans probably is still too great to warrant identification of physiologic races

TABLE 2.—*Parasitic behavior of isolates of Rhizoctonia solani as determined on the basis of direct inoculation of under-ground stems of 6 varieties of beans*

Isolate		Number of plants inoculated	Degree of relative susceptibility	Coefficient of variability	Number of plants inoculated	Degree of relative susceptibility	Coefficient of variability
Accession number[a]	Source						
		Currie's Rust Proof			Bountiful		
P-7	Grand Forks, N. D.	95	20.0	35.9	51	22.0	40.0
P-27	Laramie, Wyo.	139	28.0	23.5	91	27.5	32.5
P-116	Baton Rouge, La.	188	29.5	17.5	89	30.0	30.8
P-100	Baarn, Holland	167	30.0	23.9	78	25.0	38.0
P-105	Chwaliszewo, Poland	195	30.0	20.1	63	51.0	7.4
P-85	Ste-Anne de la Pocatière, Que.	141	33.0	16.4	77	25.0	46.6
P-20	Dilworth, Minn.	196	38.0	8.9	73	34.0	34.3
SB-33	Oslo, Minn.	207	38.5	23.3	89	41.0	27.2
SB-42	Michigan	199	57.0	14.1	85	46.5	13.0
SB-43	Ohio	198	64.5	10.0	88	72.0	14.5
SB-50	East Grand Forks, Minn.	192	84.5	5.8	90	82.0	12.2
SB-45	California	194	89.5	5.5	88	91.5	7.1
SB-13	Chaska, Minn.	213	91.0	1.8	92	94.0	0.6
		Pencil Pod Black Wax			Kentucky Wonder		
P-7	Grand Forks, N. D.	54	17.5	32.6	57	26.0	18.0
P-27	Laramie, Wyo.	118	27.0	44.4	96	19.0	35.3
P-116	Baton Rouge, La.	98	28.5	42.0	108	30.5	29.5
P-100	Baarn, Holland	103	25.0	40.8	94	33.0	28.3
P-105	Chwaliszewo, Poland	75	38.5	21.5	59	61.0	23.9
P-85	Ste-Anne de la Pocatière, Que.	93	20.0	48.3	92	28.0	28.7
P-20	Dilworth, Minn.	89	33.5	30.2	99	29.0	77.3
SB-33	Oslo, Minn.	92	41.0	24.8	99	48.0	32.5
SB-42	Michigan	92	53.0	9.8	97	58.5	22.9
SB-43	Ohio	100	63.0	8.9	88	77.0	12.3
SB-50	East Grand Forks, Minn.	88	79.0	9.8	89	98.5	1.3
SB-45	California	87	85.0	15.3	91	98.0	2.4
SB-13	Chaska, Minn.	96	80.5	11.2	87	98.0	2.1
		Giant Stringless			Manchu Soybeans		
P-7	Grand Forks, N. D.	52	18.5	40.7	69	20.0	44.1
P-27	Laramie, Wyo.	91	19.5	28.4	80	23.5	46.1
P-116	Baton Rouge, La.	83	30.0	31.9	137	38.0	13.8
P-100	Baarn, Holland	84	29.0	30.8	94	21.0	32.9
P-105	Chwaliszewo, Poland	68	35.5	19.0	124	27.5	19.5
P-85	Ste-Anne de la Pocatière, Que.	93	25.0	36.6	120	27.5	20.9
P-20	Dilworth, Minn.	96	31.0	27.3	117	27.5	23.5
SB-33	Oslo, Minn.	80	39.0	21.4	127	25.5	80.5
SB-42	Michigan	96	57.0	10.3	125	29.0	22.0
SB-43	Ohio	90	62.0	25.2	136	54.5	22.8
SB-50	East Grand Forks, Minn.	88	78.0	9.7	118	87.0	19.9
SB-45	California	82	91.5	8.1	126	91.0	18.5
SB-13	Chaska, Minn.	81	82.0	6.4	128	93.0	5.4

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[a] P = Potato isolate; SB = Sugar-beet isolate.

of the isolates from the data presented in table 2. Increase in number of plants inoculated and more accurate control of environmental conditions may reduce variability in virulence between successive tests to such an extent that this method of inoculation may become useful.

Other Host Plants

The parasitic behavior of the isolates tested on beans was determined for celery, pea, tomato, cabbage, and carrot. This work was not so extensive as that with varieties of beans; hence, the results will be summarized briefly.

On celery and carrots only 2 types of infection were noted, viz.,

Type 0 = no infection produced. Type 1 = infected.

Celery plants were considered infected when wilted, or if not wilted, when the vascular system was discolored. The presence or absence of discoloration was determined by cutting each plant at the point of inoculation and noting the condition of the vascular area. Noninfected stems were distinctly white. Infection in carrots was determined by the presence of decay

Fig. 1. Differences in size and abundance of sclerotia produced on carrot roots by isolates of *Rhizoctonia solani*. A. Potato isolate, P–7. B and C. Sugar-beet isolates, SB–33 and SB–43, respectively.

TABLE 3.—*Parasitic behavior of isolates of Rhizoctonia solani as determined on the basis of direct inoculation of under-ground stems of celery, peas, tomatoes, cabbage, and carrots*

Isolate[a]	Celery			Pea			Tomato			Cabbage			Carrot		
	Tests	Plants inoculated	Degree of relative susceptibility	Tests	Plants inoculated	Degree of relative susceptibility	Tests	Plants inoculated	Degree of relative susceptibility	Tests	Plants inoculated	Degree of relative susceptibility	Tests	Plants inoculated	Degree of relative susceptibility
	No.	*No.*	*Per cent*	*No.*	*No.*	*Per cent*	*No.*	*No.*	*Per cent*	*No.*	*No.*	*Per cent*	*No.*	*No.*	*Per cent*
P-7	0			2	39	14.0	1	35	50.0	0			1	26	4.0
P-20	2	25	4.0	3	64	27.5	2	63	74.0	1	15	0.0	1	28	11.0
P-27	2	25	0.0	1	17	50.0	1	25	62.0	1	17	0.0	0		
P-85	1	12	8.3	3	61	31.0	2	60	58.5	1	15	0.0	1	28	14.0
P-100	2	25	20.0	2	41	34.0	2	60	40.0	1	15	0.0	1	27	7.0
P-105	2	24	8.3	3	66	19.5	2	62	50.0	1	14	0.0	1	25	4.0
P-116	2	24	20.8	3	60	34.0	2	62	79.0	1	15	0.0	1	27	4.0
SB-13	2	25	100.0	3	56	99.5	2	53	88.5	1	15	100.0	1	23	100.0
SB-33				3	58	41.5	2	54	32.5	1	15	46.5	1	24	87.0
SB-42	2	25	32.0	3	58	59.5	2	59	34.0	1	15	3.5	1	24	42.0
SB-43	2	24	37.5	3	59	67.5	2	51	74.5	1	16	75.0	1	23	96.0
SB-45	2	26	34.6	3	61	87.0	2	51	73.5	1	15	66.5	1	25	40.0
SB-50	2	25	100.0	3	57	66.5	2	59	91.5	1	15	100.0	1	27	100.0
Control	2			3	62	0.0	2	58	0.0	1	15	0.0	1	26	4.0

[a] P = Potato isolate; SB = Sugar beet isolate.

in the roots. The same scheme used for beans was followed for peas, tomatoes, and cabbage. The degree of relative susceptibility was determined for all these crops by the same method as described for varieties of beans.

The parasitic behavior of the 13 isolates to these 5 different hosts is given in table 3.

It is important to note that none of the potato isolates were pathogenic to cabbage. This corroborates, in general, the results of Gratz (1) and Wellman (6). Carrots appear to be markedly more resistant to the potato isolates than to the sugar-beet isolates. On carrots and peas a number of the isolates produced sclerotia, some producing large and others small sclerotia (Fig. 1).

From the foregoing discussion it appears that these facts may be helpful in the formulation of a program for the differentiation of physiologic races of *Rhizoctonia solani.* The high degree of specificity of potato and sugar-beet isolates to cabbage should be given considerable attention. Intensification of study of the pathogenicity to carrots may bring out marked differences between isolates. Additional differentiation of physiologic races probably can be accomplished by the use of sclerotial production on carrots and peas.

DISCUSSION

Previous studies (3, 4) have demonstrated that some potato isolates of *Rhizoctonia solani* are pathogenic to older sugar-beet roots, although many are nonpathogenic. Gratz (1) and Wellman (6) found that *Rhizoctonia* from potatoes did not cause wire stem of cabbage. Similarly, Lauritzen (2) and Tervet (5) report that the potato isolates of this pathogen tested were nonpathogenic to turnips and flax. Thus, in a plan for determination of physiologic races the above crop plants would be useful as possible differential hosts.

One of the outstanding features of these studies is the degree of variability among the results, which serves to emphasize the complex nature of the problem. Therefore, any classification of physiologic races of *Rhizoctonia solani* should consider not only the relative virulence of the isolate tested, but also the degree of variability of virulence between successive experiments.

SUMMARY AND CONCLUSIONS

The study herein reported was an attempt to evaluate 2 inoculation methods for the determination of physiologic races of *Rhizoctonia solani* on the basis of pathogenicity to a number of crop plants. A comparison was made of damping-off experiments and direct-inoculation of underground stems of older plants.

The results for individual isolates from the damping-off data in successive tests were more variable than were those from direct inoculations. The latter method appears most promising, if a large number of plants are inoculated with each isolate to be tested and if environmental conditions can be accurately controlled.

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WHITE RUST OF SPINACH

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HISTORY AND IMPORTANCE

White rust of spinach, *Spinacia oleracea* Mill., caused by an *Albugo* was first found on the market in March, 1937, in a carlot of spinach received at New York, N. Y., from the Winter Garden[4] region of Texas (22). During the next four weeks the disease was observed in varying amounts in a number of other carlots from the same region. In one carlot as many as three-fourths of the plants examined in baskets selected at random were found to have from 1 to 3 leaves per plant slightly to severely affected.

At the beginning of the 1937–1938 spinach-growing season when one of the writers assumed his duties at the Texas Agricultural Experiment Station, Substation 19, at Winter Haven, the white rust was found in destructive form in the Winter Garden region and other parts of the State and became by far the most important disease of the 1937–1938 crop (8). It was present in every field examined. In many fields all of the plants were infected. In a few cases the crop was so severely damaged that the fields were left uncut. The loss for the entire area appears to have been in the neighborhood of at least 25 per cent of the total crop.

White rust was likewise observed on the New York market from time to time throughout the same season (23). In the reports made by the Fruit and Vegetable Inspectors of the U. S. Department of Agriculture covering the inspection of 241 carlots of Texas spinach at New York City during the general period extending from mid-December, 1937, to mid-February, 1938, white rust was noted in 36 carlots. The number of plants affected in these lots varied from 2–5 per cent to 30–35 per cent with an average infection of nearly 11 per cent of the total. Affected plants had for the most part from 1 to 4 outer leaves severely spotted.

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[4] Dimmit, Maverick, Uvalde, Zavala, and Frio counties in a general way comprise the so-called Winter Garden region of the State. Within that area is found approximately half of the acreage devoted to market spinach in the United States.

Comparatively little is known regarding the earlier history of white rust of spinach. Undoubtedly, it was in the region for several seasons prior to becoming of commercial importance there.[5] So far as the writers know the only previous record of a white rust of spinach is a report in the Plant Disease Survey files by J. S. Cooley of the appearance of a species of *Albugo* on spinach in Augusta County, Virginia, in 1910.

SYMPTOMS

The symptoms of white rust on spinach are similar to those of the white rust of other plants. The disease is at first readily recognized by the presence on the leaves (Fig. 1) of the characteristic white blister-like pustules that constitute the conidial sori of the pathogen. The sori may be either roughly circular or irregularly elongated; their dimensions vary from ½ mm. or less to several millimeters. Although usually confined to the lower side of the leaf the sori are at times found on the upper side (Fig. 1, C). Ordinarily, the pustules first appear near the periphery of the outermost leaves (Fig. 1, B) and later over much of the underside of all leaves (Fig. 1, B, D–G). The sori may be scattered or they may occur abundantly in groups so that several hundred or more are found on a single leaf.

The leaf tissues adjacent to the sori and those on the upper side of the leaf directly opposite the sori (Fig. 1, A and C) soon become chlorotic. In severe infections these tissues die thus forming round necrotic spots. In severe cases the entire leaf may turn brown.

THE CAUSAL ORGANISM

There have been comparatively few authenticated reports of the occurrence of species of *Albugo* on members of the Chenopodiaceae.

The files of the Plant Disease Survey of the Bureau of Plant Industry contain a report referring to *Albugo candida* (Pers.) Kuntze an *Albugo* found by J. S. Cooley in 1910 in Augusta County, Virginia, on spinach. Through the courtesy of S. A. Wingard it has been ascertained that no specimen representing this collection is now available. As already noted this is the only known record of *Albugo* on spinach previous to the recent occurrence in Texas.

Barker and Neal (1, p. 31) listed an *Albugo* on *Chenopodium album* from Coahoma County, Mississippi, as *A. candida,* and Martin (9, p. 424) included this report in his compilation. No trace of any specimen to authenticate this report has been obtainable. There are also reports in the Plant Disease Survey files of this species on *Chenopodium* from Texas, but no specimens are extant in so far as is known. McCurry and Hicks (12) listed *A. candida* as

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[5] The late Dr. J. J. Taubenhaus in reply to an inquiry by Dr. H. A. Edson stated in a letter of April 13, 1937, that during both 1935 and 1936 he had collected specimens of spinach from the Winter Garden region that had been affected with *Albugo* sp., but that the infection had been rather rare.

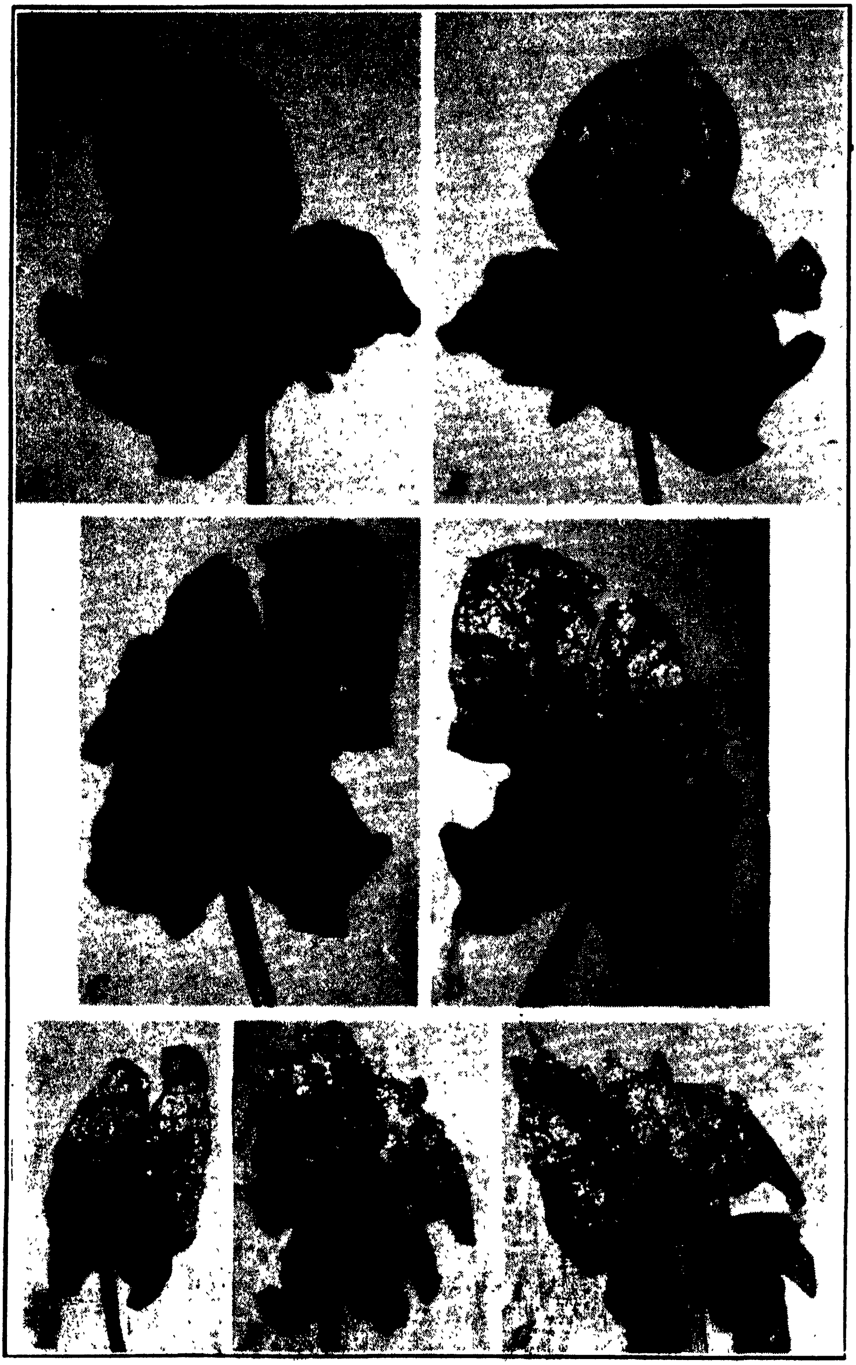

FIG. 1. White rust of Texas spinach. A and C. Chlorotic areas on upper surface of leaf opposite sori. B, D, E, F, and G. Sori of *Albugo occidentalis* on lower surface of leaf (A and B same leaf; C and D same leaf). Reduced.

occurring on *Chenopodium* sp. at Indian Head, Saskatchewan, in 1925, but no material has been available for study. In none of these cases does it appear that any critical study was made of the fungi involved, particularly as to the character of the oospores.

Comparative studies clearly indicate that *Albugo candida* is not the species occurring on *Spinacia oleracea* in Texas. The species is confined to cruciferous hosts, and differs from the spinach pathogen in a number of important morphological particulars. As described and illustrated by Wilson (24, p. 64) the conidia of *A. candida* are globular with uniform, thin walls, and the oospores are verrucose, "or with low, blunt ridges which are often confluent and irregularly branched." In other words, the species falls within the first of the two groups based on oospore surface markings into which Wilson divides the genus. As will be noted hereafter, the surface pattern of the oospores readily places the spinach fungus in the second group.

Albugo bliti (Biv.) Kuntze also has been used indiscriminately as a designation for specimens collected on various members of the Chenopodiaceae, apparently without a careful study of the fungus present. In several cases, and perhaps in most of those under review up to this point, conidia only were present, and this circumstance practically precluded an exact specific determination.

Berlese and de Toni (2) reported *Atriplex* sp. as a host of *Albugo bliti*; but, as Wilson (24 p. 82) noted, nothing further was then known about the record, nor have the writers been able to trace it further. Martin (10, p. 37) reported the species on *Chenopodium* sp. from New Jersey and on *C. album* from Virginia. Upon examination of the original record card it was found that the New Jersey report merely listed *Albugo* on "pigweed," a common name interpreted as *Chenopodium* in compiling the summary. The specimen, when located in the Mycological Collections of the Bureau of Plant Industry, was found to have been correctly named and filed as *A. bliti* on *Amaranthus retroflexus*. No trace of either records or specimens has been found to substantiate the Virginia report. Similarly, Bisby, Buller, and Dearness (3) and Conners (5) list the species on *Monolepis nuttalliana*. Through the kindness of J. Dearness, of London, Ontario, a specimen of *Albugo* on this host was received for study. Fortunately, a few oospores were present and, as will be noted from the illustration (Fig. 2, I), the finely reticulated epispore indicates that the fungus is *A. occidentalis*. This species is discussed more at length hereafter.

Pammel in 1891 (13) mentioned a white rust of sugar beet in Iowa, which he tentatively assigned to *Albugo* (*Cystopus*) *bliti*, although oospores were not found. In a second paper (14, p. 102) the same organism was again reported on a beet leaf, with oospores still lacking. Later accounts by Brock (4), Gilman and Archer (6), Massee (11), Raeder (15), Selby (17), Stevens and Hall (19), Taubenhaus (20), and possibly others of the occurrence of *Albugo* on *Beta* are all apparently based on the collections listed by Pammel. I. E. Melhus has informed us that he has been unable to locate either the

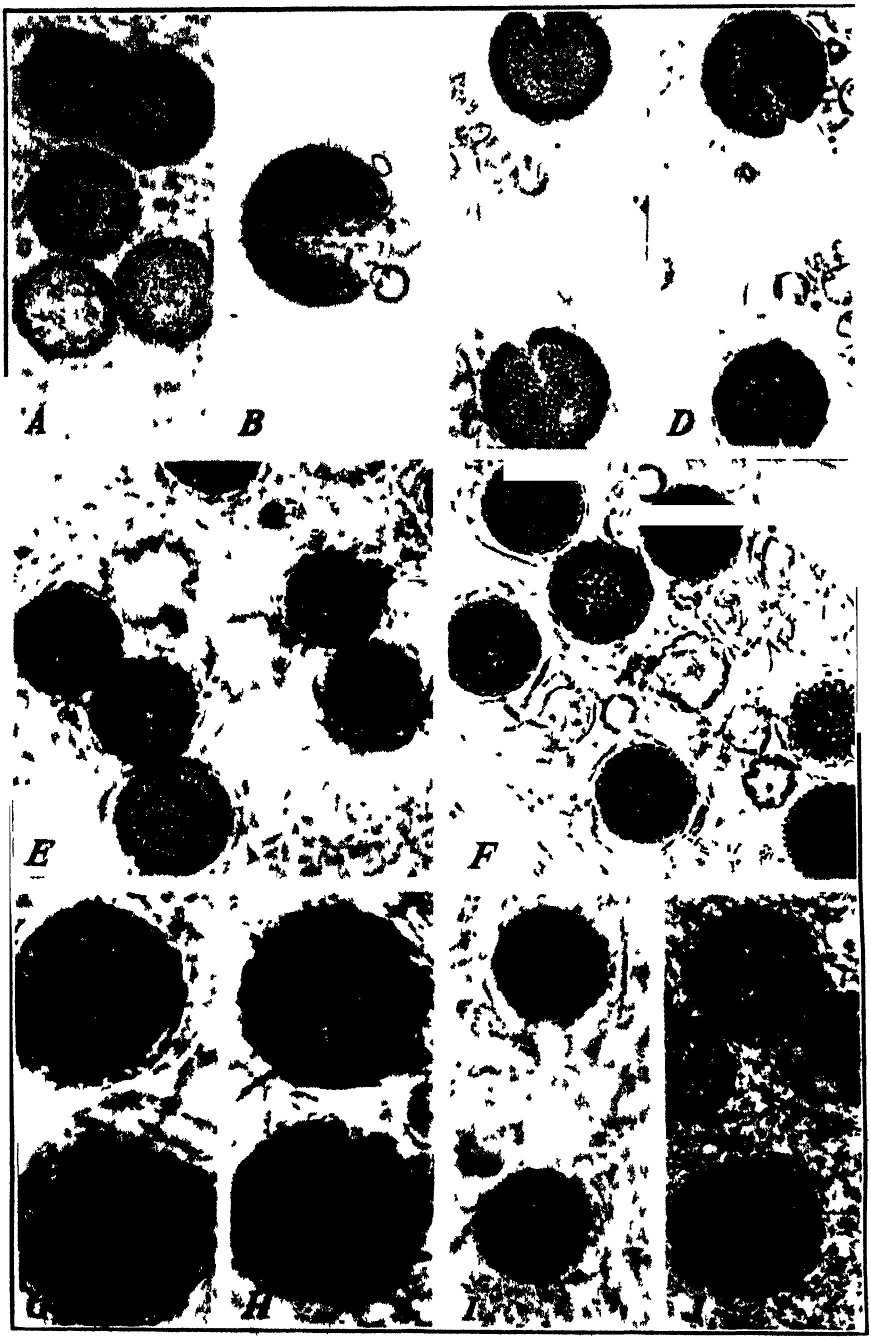

Fig 2 *Albugo* oospores. A and B. *A. occidentalis* from spinach, Texas C and D *A. occidentalis* from *Chenopodium capitatum*, ex Type. E. *A. occidentalis* from *Chenopodium capitatum*, Wyoming. F. *A. eurotiae* from *Eurotium*, Russia. G and H. *A. bliti* from *Amaranthus*, W. Virginia. I. *A. occidentalis* from *Monolepis*, Canada. J. *A. bliti* f. *achyranthes* from *Achyranthes*, Japan. × 300. Photomicrographs by M. F. L. Foubert

Pammel specimens or, in fact, any *Albugo* material on *Beta,* in the herbarium of the botany department at Ames.

Albugo bliti is a member of the second group of *Albugo* species as recognized by Wilson (24), and is characterized by oospores with a reticulate epispore in contrast to those with the tubercles or ridges of the first group. In *A. bliti* the oospore reticulations are very definite, large-meshed, with areolae 6–8 μ in diameter without central elevations (Fig. 2, G, H, J). The conidia are hyaline, elliptic to globular, and equatorially thickened. The character of the epispore and other minor features serve to differentiate this species from that actually found on the Texas spinach material.

Tranzschel and Serebrianikow (21) in 1911 issued as No. 101 of their exsiccati series, "Mycotheca Rossica," a specimen of an *Albugo* on *Eurotia ceratoides* of the Chenopodiaceae, which was designated as *Albugo eurotiae* Tranzsch., n. sp., but without description. This binomial was recombined as *Cystopus eurotiae* (Tranzsch.) Sacc. and Trott. in the Sylloge Fungorum **24**: 34, 1926, and a description supplied. From the description of Saccardo and Trotter, alone, this species is not clearly separable from the fungus found on spinach, though the conidia of *A. eurotiae* are given as slightly larger and its oospores as a few microns less in diameter than those of the Texas fungus.

An examination of material of *Albugo eurotiae* found in the Mycological Collections reveals a definite difference in the character of the epispore of the two forms. In the case of *A. eurotiae* the reticulations are coarser (Fig. 2, F), so that the areolae are wider (about 4 μ) than in the spinach fungus, and they are somewhat deeper. The species would appear to approach *A. platensis,* in so far as oospore markings are concerned.

Savulescu and Rayss (16) described a form found on *Ceratocarpus arenarius* under the name of *Cystopus eurotiae* (Tranzsch.) Sacc. and Trott. forma *ceratocarpi* Savul. and Rayss. This new form, collected in Roumania, was said to have all the morphological characters of the species. It need not, therefore, be further considered here.

Wilson (24, p. 80), in his monograph of the genus *Albugo,* described, as new, *Albugo occidentalis,* selecting as type a specimen at the New York Botanical Garden on *Chenopodium* (*Blitum*) *capitatum* collected by L. M. Underwood and A. D. Selby in Colorado in 1901, and originally referred to *A. bliti.* A second specimen on *Chenopodium rubrum* collected by Kelsey in Montana was also referred to the new species. Wilson, in his discussion of his new species (24, p. 82), mentioned Pammel's report of *Albugo* on beet in such a way as to suggest that he felt it should be referred to *A. occidentalis.* In a later paper (25) he listed "*Albugo? occidentalis* on *Beta vulgaris*" again, apparently on the basis of Pammel's reports. Further than these two original specimens only one additional collection has been referred to Wilson's species in the intervening years. This was issued by Solheim (18) as No. 101 of his exsiccati, "Mycoflora saximontanensis exsiccata," and the host is again *Chenopodium capitatum.*

Through the courtesy of F. J. Seaver, Curator of Fungi, New York

Botanical Garden, it has been possible to examine for comparative purposes a portion of Wilson's type, and a specimen of Solheim's collection has also been available from the Bureau of Plant Industry collections.

The conidia of *Albugo occidentalis* are described as discoid, hyaline, with an equatorial thickening, the contents yellow, 14–20 × 8–16 μ. Conidia of the spinach form are 16–22 × 13–15 μ, fairly well within the range established by Wilson for his species. The discoid character is evident when a dry mount is examined. It is noted that the conidia of the Texas material are definitely hyaline. There is a tendency, however, for the conidial masses to become somewhat yellow with age and it appears reasonable to assume that the yellow conidial content noted by Wilson is merely a matter of age and not a specific peculiarity. The specimens studied by him had been lying in an herbarium for some 5 or 6 years before being examined critically.

While the conidia of the two correspond fairly well, it is the oospores that give a more exact determination. Wilson (*l.c.*) describes them as "globular, 50–60 μ, yellowish-brown, very closely and shallowly reticulate, areolae about 2 μ." Although comparatively few oospores were present in the portion of the type studied by the authors, those found were typical, measuring 50–55 μ. The areolae were also quite typical (Fig. 2, C, D). Oospores found in abundance in the leaves of the Texas collections (Fig. 2, A, B) were essentially identical with those of the type of *Albugo occidentalis,* as well as with those of the Solheim specimen (Fig. 2, E), ranging in diameter from 48–66 μ, with typical areolae. A perusal of the descriptions of other *Albugo* species described since the appearance of Wilson's monograph does not reveal any that are at all suggestive of the species here under discussion. The use of the binomial *Albugo occidentalis* Wilson for the spinach white-rust fungus appears to be entirely in order.

In the light of the rather sudden appearance of this fungus on spinach it is possible that a specialized form or physiologic race may have arisen in this instance. This can be only suggested at this time, however, since the inoculation experiments with the fungus on spinach and on other chenopodiaceous hosts, necessary to test such an hypothesis, have not been made. It is of interest to note at this time, however, that 3 varieties of sugar beets interplanted with spinach remained free of *Albugo,* while the spinach plants all became infected.

SUMMARY

White rust of spinach, caused by an *Albugo* was first noted in March, 1937, on the New York market in shipments from the Winter Haven section of Texas. The disease was destructive throughout this region during 1937–38. Characteristic lesions occur on the leaves, usually on the lower surfaces, the infected leaves tending to become chlorotic and finally brown. Although *Albugo candida, A. bliti,* and *A. eurotiae* have been reported on various members of the Chenopodiaceae, these species are shown to differ from the spinach fungus, which is assigned to *A. occidentalis* Wilson.

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A PYTHIUM ROOT ROT OF CUCURBITS

MANFRED GOTTLIEB AND KARL D. BUTLER

(Accepted for publication December 1, 1938)

INTRODUCTION

A watery root rot of watermelons, honeydew melons, quil muskmelons, and crookneck squash has been found in Arizona. It was first noticed in July, 1935, in a badly diseased watermelon field in the Salt River Valley, where over 20 per cent of the plants were infected. Still later in the season the disease had become more severe, until 40–50 per cent of the plants had been destroyed. Only a few hills of the honeydew melons, muskmelons, and squashes had been planted in this field, but the presence of the disease on them, also, was unmistakable. In 1936, watermelons were planted in the same field; this resulted in even more severe losses. Also, the disease caused considerable loss in 1935, in a 5-acre watermelon field about 2 miles distant. In both fields corn had been interplanted, yet no trace of infection could be found on these plants. From many isolations made from lesions on diseased cucurbit roots, a phycomycetous fungus was regularly isolated. A brief description of the disease and its causal fungus follows.

THE DISEASE

The disease is characterized by rather sudden wilting of the infected plants in the field; some plants wilt before the melons begin to mature but the wilting becomes more pronounced later in the season. Excavated roots of affected plants showed light-brown, watery, depressed lesions, which varied in size from 3–15 mm. in diameter to a very large lesion, which sometimes almost entirely covered the root. It was not uncommon to find several lesions that had resulted from separate infections that had coalesced to form large, depressed, rotted areas. In the late stages of development the rotted roots assumed a watery, soft-rot appearance typical of similar diseases caused by species of Pythium and Phytophthora (Fig. 1, A). In a few cases indefinite lesions were found on the stems, especially on stems in direct contact with the wet soil. Also, as has already been described by Brown and Evans (1) for *Phytophthora cactorum,* the fungus studied herein was commonly found to be causing fruit rot in the field.

The soil in the fields in question was a medium heavy loam and rather poorly drained, so that with heavy irrigations, plus the usual late summer rains, the soil remained very wet for long periods. This high-moisture condition, occurring concomitantly with high summer temperatures, appeared to be ideal for the development of the disease. Possibility of introduction of the fungus with seed was suspected, but seed samples from which the plants were grown were not obtainable.

ISOLATIONS

Numerous isolations of a phycomycete from lesions on roots of diseased plants were obtained at different intervals. Similar cultures from small

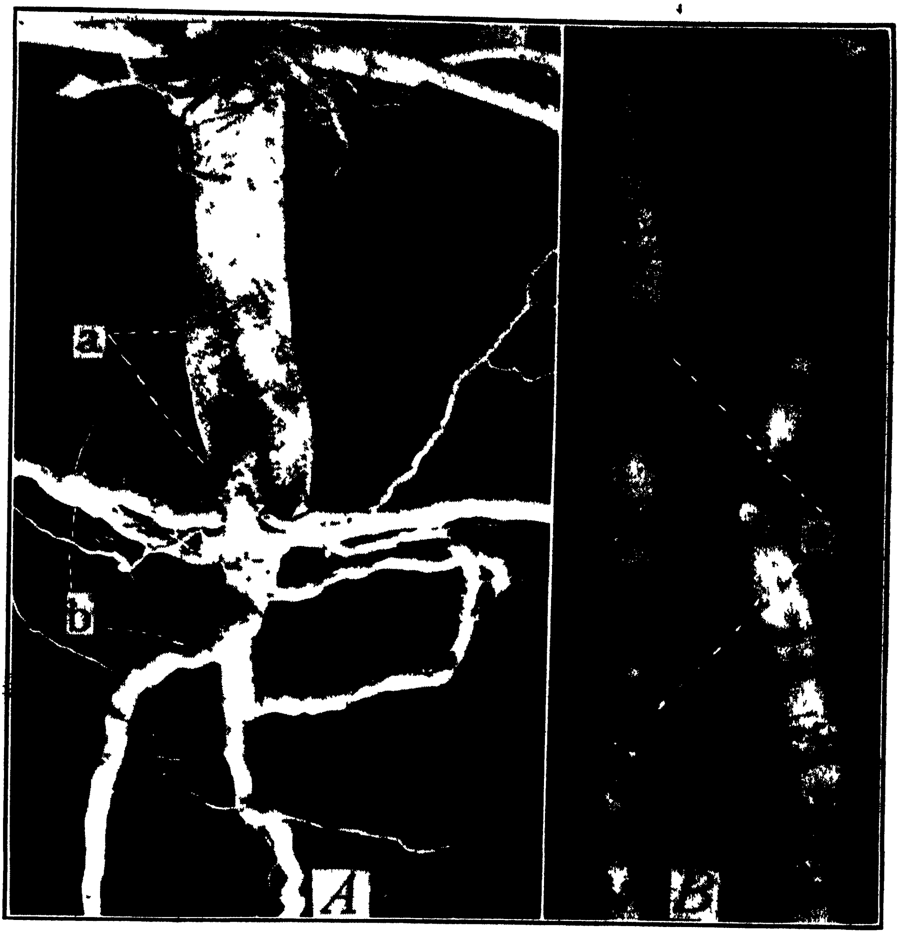

FIG. 1. *A*. Decayed root of mature watermelon plant, natural infection: a, Large, coalesced lesion on primary root; b, rotted secondary roots. *B*. Lesions, n, on secondary roots of watermelon induced by inoculation with *Pythium aphanidermatum*.

pieces of the vascular elements, as well as from the water-soaked, cortical, noncutinized, and nonlignified tissues, were obtained without difficulty, except for occasional bacterial contaminations. Nevertheless, from many attempts only one culture showed any other than the one fungus regularly found, and this was a species of *Fusarium* that was mixed with a culture of the usual phycomycetous isolate. Inoculations with this fungus were all negative. Isolations also were made from rotted fruits, where oospores of the causal fungus were formed in great profusion both within and outside the host tissues. Although this fungus causes a very similar fruit rot to the Phytophthora rot previously mentioned, the former has never been found producing sporangia (conidia) in nature.

GROWTH, REPRODUCTION, AND IDENTIFICATION

On artificial culture media the causal fungus grew with great luxuriance.

Oogonia usually appeared in large numbers, inversely to the rate of growth. Growth is much more luxuriant on cornmeal than on potato–2 per cent dextrose agar on which oogonia and antheridia developed in 6 days and 3 days respectively. Inasmuch as growth on these media did not produce sporangia, transfers of sterile mycelium to the following substrata and media were grown in the incubator at 27° C. and at room temperature, 27–32° C.: Steamed watermelon leaves and stem extract (200 g./l.), nutrient broth, potassium nitrate (.01 per cent) broth and agar, pea broth (4), sterile distilled water, and tap water, as well as the following fruits in moist chambers: Apples, cucumbers, squashes, cantaloupes, watermelons, and pears.

In each case, before oogonial formation, mycelial wefts were taken from potato-dextrose agar, washed 3 times in successive changes of sterile distilled water, and then transferred to the media and substrata described above. Even though these cultures were maintained and examined almost daily for 3 months, no sporangia developed. At the same time, oogonia of different sizes appeared in all of the cultures to a greater or less degree. Some measurements of oogonia are given in table 1.

TABLE 1.—*Size, in microns, of oogonia of Pythium sp. on various substrata*

Substrata	Number measured	Minimum	Maximum	Mean
Sterile carrot plugs	40	18.5	27.25	22.94
Sterile bean pods	75	18.2	29.60	27.01
Mature watermelon fruits	150	18.4	27.43	24.03
Cucumber fruits	60	18.3	28.16	25.01
Tomato fruits	45	23.4	37.39	29.21
Squash fruits	25	18.2	27.36	23.61
Sterile sweet-potato plugs	50	18.5	27.35	22.64
Potato–2 per cent dextrose agar	100	18.3	26.89	22.42
Yellow cornmeal plates	50	18.7	27.92	23.64

The number of oogonia measured is a relative index of their abundance on the different substrata. From these results and from the general appearance of the growth of the fungus the writers were convinced they were working with a species of *Pythium*. And from the work of Drechsler (2), Edson (3), and Mathews (5), it appeared that the fungus was *Pythium aphanidermatum* (Edson) Fitzpatrick. The identification was verified by W. C. Coker who stated: "Cultures on hemp seed, wheat endosperm, and rice in water produced plentiful sporangia." Attempts to produce the sporangial stage on these and other substrata still resulted in recurrent failure under Arizona conditions until the pH of the water used was lowered to about 5.4; even then, the sporangial stage was exceedingly evanescent, being present for only about 2 days.

In sterile distilled water the mycelium assumes many peculiar forms similar to those reported by Leonian (4) and Tucker (6) for various *Phytophthora* spp., such as swollen hyphal tips, tuberculate branching, chlamydospore-like swellings, and hyphal knots. These structural changes are even more evident in water when a piece of infested tissue is retained with the mycelium, and bacterial contamination follows. To determine the effect of temperature on growth and possible sporangial production, the fungus was grown on various media at 5, 12, 26, 37, and 45 degrees C. No growth was apparent at 5°, and the growth was very slight at 45°, with the most luxuriant growth at 37° C. Sporangia were not found in any case.

The loss of the great vegetative vigor of this fungus, following the original isolations, has been striking. During the past 16 months its vigor has steadily decreased and oogonial formation in culture has correspondingly declined. Although oogonia are still formed abundantly, the number of mature oospores has diminished considerably. Successive passages of the fungus into cucurbitaceous fruits somewhat increases its vigor, but for only a short time.

Oospore germination in sterilized tap water in watch glasses was observed from material taken from 2-week-old potato-dextrose and malt-agar cultures. The percentage germination was very low. Furthermore, cultures on various media and most substrata almost invariably lost viability after 3 months of continuous growth on the same medium. Attempts to obtain germination of oospores from these old cultures have always failed. Such oospores, therefore, are apparently either dormant or dead.

INOCULATIONS

Fifteen pots of watermelon seedlings 3 weeks old, which had been planted in sterilized soil, were inoculated by placing pieces of 4-day-old cultures on yellow cornmeal plates within the soil in each pot. This was done in late summer, 1935, when the prevailing greenhouse temperatures were exceedingly high and the soil was wet. Three days after inoculation nine seedlings had wilted. Upon examination the roots of these seedlings were found to be severely rotted, showing that under favorable conditions the fungus is highly virulent. Similar inoculations were made at the same time in Icicle radishes in the greenhouse. Of 10 plants inoculated, 3 wilted. The causal fungus was easily reisolated from all wilted plants.

Six mature watermelon plants growing in a plot on the campus of the University of Arizona were inoculated by the same method on September 31, 1935. The inoculum was placed adjacent to uninjured roots in each case. The roots were then carefully covered with soil and the plants were watered heavily for a week. In anticipation of frost the roots were carefully excavated and examined 16 days later. There were definite, wet, sunken lesions on 12 secondary roots (Fig. 1, B). The lesions were identical to those found on the originally diseased roots. These experiments show definitely that the fungus causes a wet root rot and that penetration into the suscept by the fungus can take place through sound, uninjured tissues.

In July, 1936, more inoculations were made in the field with 112 1-month-old watermelon plants. All results were negative. It is possible that the *pathogenicity of the fungus was attenuated after continued growth on culture media, or that other factors were not conducive to infection.*

An isolation (July, 1937) from the wilted watermelon plant yielded an identical fungus that, on inoculation into 35 pots of 3-week-old watermelons (var. Iowa King), resulted in 22 wilted plants within 5 days. Again, the causal organism was readily reisolated from the roots of the dead plants.

Apples, cantaloupes, carrots, cucumbers, eggplants, grapes, summer squash, sweet potatoes, and tomatoes, artificially inoculated and maintained in a moist chamber, decayed rapidly. Although most of these were inoculated by incisions, wounding was not necessary to fungal invasion. The response to the fungus was similar to that reported for eggplant fruits by Dreschler. Although inoculations by incision into Irish potato tubers invariably resulted in a watery "leak," infection could take place only after mechanical injury. The only cucurbit that did not become infected after inoculation was the wild gourd, *Cucurbita digitata.*

SUMMARY

A watery root rot and a fruit rot of watermelons, honeydew melons, quil muskmelons, and crookneck squash caused by *Pythium aphanidermatum* are here reported.

The fungus is shown capable also of causing a rapid decay of many other fruits and vegetables.

Oospores are produced in abundance in nature as well as in culture, yet sporangial production is induced only with difficulty.

Mycelial growth is most luxuriant at about 37° C.

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HOST-PARASITE RELATIONSHIPS IN PINK ROOT OF ALLIUM CEPA L. I. THE PIGMENT OF PHOMA TERRESTRIS[1]

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(Accepted for publication April 18, 1939)

The pink coloration of onion roots invaded by *Phoma terrestris* Hans., has been considered to be the principal symptom, and, accordingly, the chief diagnostic index of pink root.[3]

However, in some soils infested with the organism, it was observed that infected onion roots occasionally failed to reveal the characteristic pigmentation of pink root except in portions of certain turgid and healthy appearing roots. In this latter case, these affected regions frequently revealed a faint rhodonite pink[4] coloration, which could be detected only after a minute examination. Dead and dying roots instead of appearing rhodonite pink to spinel red[3, 4] as expected, approached a Mars yellow[4] in hue. A microscopic examination of such tissues usually revealed the presence of those bodies previously described by Hansen[3] as "pycnidial primordia," which appear to be a constant manifestation following invasion by *Phoma terrestris*. In addition, platings from such tissues yielded colonies of the organism.

From such observations, it follows that the lack of the typical pink to red hues in roots does not necessarily indicate that the tissues are not invaded by *Phoma terrestris*.

Early in the study of this organism, it was observed that temperature and moisture variation apparently had little to do with the nature of the pigment expression. *Phoma terrestris* was grown for months at a time on different types of standard media, which in some instances were supplied with varying amounts of sugars and salts, to ascertain whether or not such nutrient variation would affect pigmentation. Although the intensity of the red-purple coloration exhibited by submerged portions of colonies in culture occasionally varied somewhat with the medium used, it was soon evident that a study of the rôle of the nutrient factors involved, would not explain the atypical pigment manifestation previously mentioned. In such studies, however, it was observed that a standard acid medium, such as prune agar, effectively prevented the appearance of the red-purple hues in cultures.

Since this observation appeared to parallel the phenomenon previously observed in diseased roots, a study of the nature of the influence of the hydrogen-ion concentration on color manifestation by the pink-root fungus was made.

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[1] Taken from a part of a thesis to be submitted to the graduate faculty of Iowa State College in partial fulfillment of the requirements for the degree, Doctor of Philosophy.

[2] The writer wishes to express his appreciation to Dr. S. M. Dietz and to Dr. I. E. Melhus of the Department of Botany and Plant Pathology, Iowa State College. He also wishes to thank Dr. L. W. Durrell of the Department of Botany, and Mr. Robert Whitney of the Department of Agronomy, Colorado State College, for helpful assistance.

[3] Hansen, H. N. Etiology of the pink-root disease of onions. Phytopath. 19: 691–704. 1929.

[4] Ridgway, R. Color standards and color nomenclature. 43 pp. Illus. Washington, D. C. 1912.

The only treatment in the literature regarding this phase of the onion pink-root condition, is the paper of Sideris.[5] This investigator, using the fungus that he believed to be the causal agent of pink root, *Fusarium bulbigenum* Cke. and Mass. (*F. cromyophthoron*), as a source of inoculum, investigated the influence of the H-ion concentration on pigment manifestation. Since apparently he did not investigate the pigmentation induced by *Phoma terrestris,* and because his data do not clarify any phase of the phenomenon observed by the writer, a reinvestigation of the whole matter was undertaken.

In order to determine whether or not the factor of hydrogen-ion concentration might influence the character of pigmentation, a series of preliminary trials was run. The materials used were thin slices of hard agar on which the fungus had been growing for three weeks which showed a pansy purple to violet carmine coloration[6] for several millimeters into the substratum, and deeply pinkened roots that had been growing in Phoma-infested soil.

Several agar slices and diseased roots were placed in each of five 250-ml. beakers, each containing 50 ml. of distilled water. Drops of 0.1 N HCl were next added until a color change was observed. At approximately pH 4.5 the color changed definitely to a chestnut or burnt sienna[6] (yellow-brown to brown shades), the pigment in roots showing a more rapid response to the H-ion shift than that in the agar slices. Neutralizing the above solutions and bringing them into the alkaline range by the addition of 0.1 N NaOH caused a change in color to the original pink-root pigmentation at approximately pH 8.5.

Believing that a more accurate study of the influence of the H-ion concentration on the color manifestation would be possible if the pigment were to be obtained in solution, steps were taken to extract the coloring substance. In this study, potato-agar mats on which the fungus had been growing for 4 weeks were used as material for extraction purposes.

Negative results were obtained with the following solvents and solutions: acetone, ethyl alcohol (95%), ether, chloroform, KNO_3 (1%), $NaNO_3$ (1%), NH_4NO_3 (1%), NH_4Cl (1%), KCl (1%), NaCl (1%), .02 N HCl, .02 N NaOH, 0.1 N NaOH, 0.1 N HCl and water. In addition none of these substances gave any extraction, even when heated to their boiling points. The above trials were next repeated using Phoma-infected roots as a source of material. Again, the results were negative.

Successful pigment extractions were obtained only by treating the agar mats with 0.1 N HCl, allowing the material to remain for 15 minutes to several hours in the acid, then pouring off this solution and adding 0.1 N NaOH. With the addition of the acid solution, the pigmented material underwent the characteristic color shift of pansy purple to chestnut,[7] and with the subsequent removal of the acid and the addition of the alkaline solution the reverse color shift took place, with the immediate indications of pigment extraction. Allowing the material to remain in the solution for from two to three hours gave the solution a deep pansy purple coloration. These trials were repeated several times with the same result.

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[5] Sideris, C. P. The effect of the H-ion concentration of the culture solution on the behavior of *Fusarium cromyophthoron* and *Allium cepa* and the development of pink-root disease symptoms. Phytopath. 19: 233–268. 1929.

[6] See footnote 4.

Portions of the colored extract were next treated with various substances in an attempt to remove or precipitate the pigment from solution. Success was arrived at only by the use of a saturated Na_2SO_4 solution, which when added to a small amount of the dissolved pigment, flocculated the coloring material, separating it from its solution after an interval of from 15 minutes to an hour.

The flocculated pigment was next removed by filtration, and was washed thoroughly with distilled water. All the solvents that were originally used in the first attempt to extract the pigment, were reemployed in an effort to redissolve the pigment flocculate. The results were wholly negative.

In order to arrive at a more accurate concept of the color shift of the pigment, as affected by pH, 50-ml. portions of the original deeply colored alkaline extract were set aside in 250-ml. beakers. Holding several samples for the control color, the contents of the 4 beakers were treated with drops of a 0.1 N HCl solution until a definite color change was observed (toward the yellow-brown or chestnut shades). This point appeared to be relatively sharp. Two of the beakers were then put aside and the contents of the other two were treated with drops of a 0.1 N NaOH solution until the original pansy purple gave indication of returning. The pH of the solutions in the beakers were then obtained by use of the Beckman acidmeter (glass electrode), with the following results:

Sample	1	—Color shifting toward yellow brown—	pH 7.00
"	2	" " " " "	pH 7.05
"	3	Original pansy purple returning	pH 7.86
"	4	" " " "	pH 7.70

From these results it appears that a far more sensitive test is obtained when the pigment is in solution rather than in the hyphae of the fungus. Subsequent tests indicated, that as the hydrogen-ion concentration dropped below pH 7 into the acid range, the color of the solutions accordingly approached a light amber-yellow. Raising the pH to 8 and above, gave a complete return of the original pansy purple coloration, which differed not at all from that of the untreated control portions.

Using the washed flocculated pigment, numerous tests were made to determine the nature of the coloring material. In a series of qualitative tests, *i.e.*, the biuret reaction, the Millon reaction, Liebermann's reaction, the Adamkiewicz reaction, the Acree-Rosenheim reaction and the Molisch test, only the last named gave a positive result. It is recognized that qualitative color reactions for protein linkages cannot be relied upon, especially where only small amounts of the material to be tested are available. For this reason the possible existence of such linkages cannot be excluded from consideration.

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[7] See footnote 4.

It was found that if the dried pigment was added to a 2 per cent solution of emulsin and incubated at 35° C., the pigment could be observed going into solution after an interval of 30 minutes. Within an hour all pigment was back in solution. Using solutions containing only the emulsin solution as a control, Fehling's test indicated the presence of reduced sugars in small amounts in the solutions containing the pigment, whereas no such evidence was found in the control solutions. This was considered as fair evidence of hydrolysis of the pigment by the enzyme emulsin. Such evidence may be interpreted to indicate that at least part of the pigment molecule is of the nature of a β-glucoside. No tests were carried out to determine the nature of the color grouping.

SUMMARY

Observations in the study of pink root of onions in Colorado, indicate that roots invaded by the causal agent, *Phoma terrestris,* may not always show the pink-red root coloration usually associated with the malady. Diseased roots were occasionally found that, although being infected with *P. terrestris,* were yellow to yellow-brown.

Cultural studies revealed that, although temperature and nutrient variation had little to do with this phenomenon, increasing the H-ion concentration produced a color change, the hue induced being comparable to that observed in nature. Preliminary trials, using diseased pink roots and agar mats on which the fungus was growing, showed a color shift from the red to red-purple shades at pH 8.5 to a yellow to yellow-brown at pH 4.5. By extracting the pigment from the fungus hyphae, using 0.1 N HCl followed by 0.1 N NaOH, the coloring material was obtained in a soluble state. Using the pigment in solution, a more sensitive test was obtained, the color shift being observed to take place within the range of pH 7.00 to 7.86.

The pigment of *Phoma terrestris* was obtained from its alkaline solution by the addition of saturated Na_2SO_4 and was returned to its soluble state only by treatment with 0.1 N HCl followed by 0.1 N NaOH. Since the material apparently is hydrolyzed by the enzyme emulsin, it appears that at least part of the molecule partakes of the nature of a β-glucoside.

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BACTERIOSIS OF TUBEROUS BEGONIA[1]

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INTRODUCTION

A leaf spot of tuberous begonia (*Begonia tuberhybrida* Voss) has been under observation and study in California for several years. It occurs in lath-house nurseries at Capitola, Colma, and Golden Gate Park in San Francisco and was observed by M W. Gardner in 1935. High atmospheric humidity, relatively high temperatures, and close contact between the plants seem to favor the development and spread of this disease. A bacterium was isolated from diseased material that, in culture, formed a yellow colony on nutrient bacteriological media. Tuberous and fibrous-rooted begonia plants were readily infected under greenhouse conditions, and the organism was recovered in pure culture.

This paper deals briefly with comparative studies of the leaf-spot organism of tuberous begonia and with proof of its pathogenicity.

REVIEW OF LITERATURE

A review of the most important of the earlier papers on bacterial diseases of begonia was recently given by Stapp.[2] In the United States, a leaf-spot disease of fibrous begonia, caused by *Phytomonas flavozonatum* (McCulloch), was recently reported,[3] while, in England, Dowson *et al.*[4] described a leaf spot of winter-flowering begonia (hybrids of *Begonia socotrana* Hook.) and determined the causal organism to be *Ph. begoniae* (Buchw.) Pape emend. Dows.

SYMPTOMS OF THE DISEASE

The symptoms of this disease consist at first of small, circular, blister-like spots, which later become very conspicuous and transparent when examined with transmitted light (Figs 1 and 2). Gradually the spots enlarge and then coalesce, forming large, blister-like, necrotic lesions. These lesions appear water soaked, while occasionally a yellow, dry ooze may be observed on dry specimens. The lesions are often very numerous and cause premature abscission of the leaf.

Invasion of the vascular system of tuberous begonia plants was observed only in the greenhouse after spraying them with a heavy water suspension

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[1] Contribution from the Division of Plant Pathology, University of California, Berkeley, California The assistance of nontechnical employees of the Federal Works Progress Administration is acknowledged.

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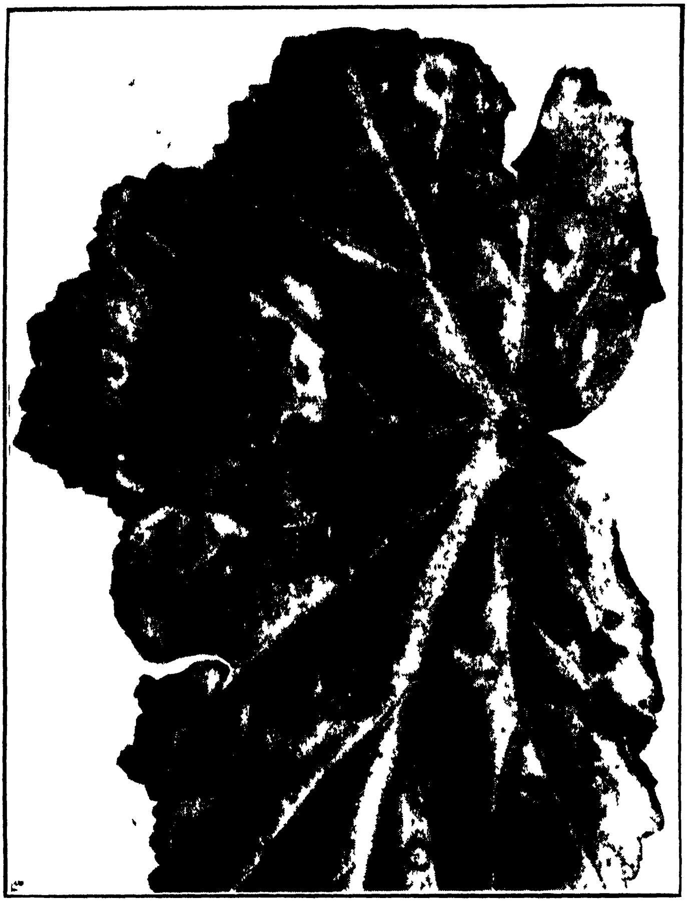

FIG. 1. Necrotic lesions of tuberous begonia leaves produced under natural conditions in a lathhouse at Colma.

of the tuberous begonia organism in a moist chamber. After 8 to 12 days, the plants were transferred to a warm greenhouse. This phase was characterized by a water-soaked appearance of the petioles, which slowly spread into the main stem with a gradual softening of all tissues. Subsequently, the entire plant collapsed. Stained microtome sections showed an abundance of bacteria in the vascular elements. In all cases, the organism isolated from infected tissues proved identical with that sprayed on the plants. These reisolates were later found, upon inoculation, to be pathogenic to tuberous begonia leaves.

INOCULATIONS

Several isolates of the bacterium were used in the inoculation tests. Healthy tuberous begonia plants were grown from both tubers and seeds, and fibrous-rooted begonia plants from cuttings and seeds. Before inoculating, the plants were placed in a moist chamber in a greenhouse having average

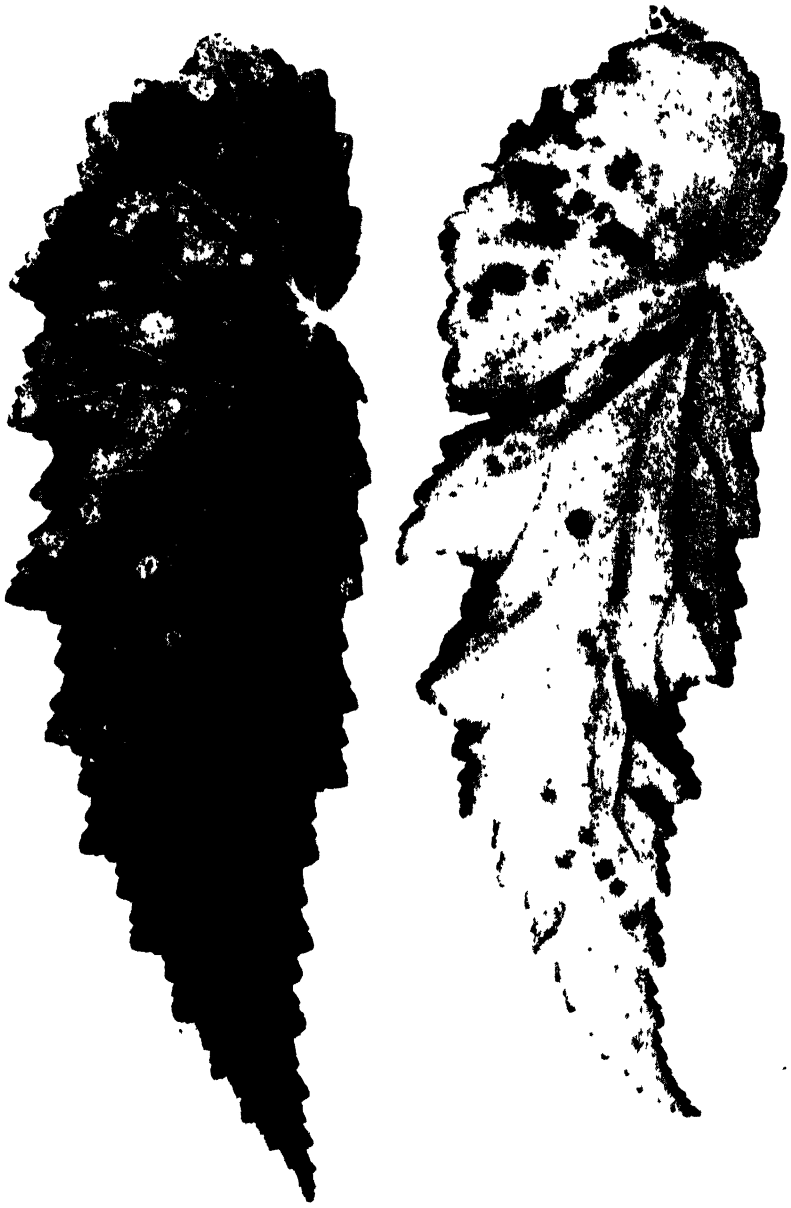

FIG. 2. Leaf spot produced on *B. lloydii* by spraying with an organism obtained from the necrotic areas of the tuberous begonia leaf shown in figure 1. A. On the upper surface. B. On the under surface 12 days after inoculation. Natural size.

temperatures of 60° F. at night and 80° to 90° during the day. After atomizing twice with a suspension of the bacteria at an interval of 24 hours, the plants were syringed with tap water in order to provide a humid atmosphere. In some instances, inoculated plants were moved to a cooler greenhouse (60° to 70° F. during the day), where conspicuous symptoms developed on petioles and stems.

The following species of tuberous begonia proved highly susceptible to this organism: *Begonia tuberhybrida, B. gracilis luminosa* HBK., and *B. lloydii* (Fig. 2). Of the fibrous-rooted type (*Begonia semperflorens* Link and Otto), infection was obtained on the varieties Christmas Cheer, Fire Dwarf, Prima Donna, Rosabelle, Salmon Queen, and Vernon; and on the species *B. erfordii* Hort., *haageana* Wats., and *schmidtiana* Regel. var. *rosea* Hort. Infection of these varieties and species was obtained also by spraying with a culture of *Ph. flavozonatum* kindly sent by Miss McCulloch.

THE CAUSAL ORGANISM

A comparative study of 6 different isolates of the leaf-spot organism from different localities in California and of *Phytomonas flavozonatum* showed no fundamental differences in morphological and cultural characters. Moreover, our data on *Ph. begoniae* was in complete agreement with those of Stapp. It should be noted that our sugar tests were made in a synthetic liquid medium to which various sugars and alcohols were added. In this medium, acid but no gas was produced from dextrose, sucrose, maltose, fructose, arabinose, glycerine, xylose, galactose, raffinose, dulcitol, mannitol, and lactose. When asparagin or Dunham's peptone base was used in sugar and alcohol tests no acid or gas was produced by our isolates or by *Ph. flavozonatum.*

Miss McCulloch's classification of the begonia leaf-spot bacterium seems to be based on the fact that the European organism is primarily a vascular parasite. She states: "Allowing for possible errors in description or technique, the bacterium of the European vascular disease is very similar in character to the bacterium causing leaf spot disease in the United States."

Since the organism causing a leaf spot of tuberous begonia so closely resembles *Ph. begoniae* described by early investigators, both morphologically and physiologically, and because of occasional vascular symptoms produced by it, it is suggested that the name *Ph. begoniae* be retained and the *Ph. flavozonatum* be considered a synonym.

CONTROL

Since the disease occurs only when air humidity is high and plants are crowded, control involves primarily a modification of present cultural practices.

Progress of the disease on artificially-infected plants was inhibited by transferring them to a greenhouse having a relatively low humidity and by providing greater spacing between plants. The organism was recovered from infected leaves that had been dried and kept in the laboratory for three months. It is, therefore, important that overhead watering or syringing should be reduced in lathhouses where the disease is known to occur.

A culture of *Phytomonas begoniae* from tuberous begonia has been forwarded to the American Type Culture Collection, Washington, D. C.

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RELATIVE EFFICIENCY OF QUASI-FACTORIAL AND RANDOMIZED-BLOCK DESIGNS OF EXPERIMENTS CONCERNED WITH DAMPING-OFF OF SUGAR BEETS[1]

E. L. LeClerg

(Accepted for publication February 13, 1939)

INTRODUCTION

In experiments involving a large number of varieties, the size of the replications may become of such magnitude that plot variability in field tests cannot be efficiently controlled. The quasi-factorial design of plot arrangement, as developed by Yates[2] for trials involving large numbers of varieties, reduces the influence of soil heterogeneity on experimental error, since the block size is smaller than in a randomized-block arrangement.

In quasi factorial experiments the replications are divided into small blocks, which, in turn, are used as a measure of error control (Fig. 1). For a detailed discussion of the various types of quasi-factorial arrangements and the methods of analysis of experimental data, the reader is referred to Yates[2] and Goulden.[3]

The relative efficiency of quasi-factorial arrangements as compared with randomized-block arrangement has been studied by Yates[2] and Goulden[4] for yield trials. They found gains in efficiency ranging from 26 to 57 per cent and 20 to 50 per cent, respectively.

In determining yields of a large number of varieties, where the influence of soil heterogeneity is pronounced and plant competition is operative for a considerable time, it is known that the experimental error is usually lower in quasi-factorial experiments than in randomized-block tests of the same size. But it has not been determined whether this would be true, also, in experiments dealing with the effectiveness of a large number of treatments for the control of damping-off and similar diseases or those in which the

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[1] The data presented in this paper were obtained in cooperative investigations by the Division of Sugar Plant Investigations, Bureau of Plant Industry, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station. Paper No. 1673 of the Journal Series of the Minnesota Experiment Station.

[2] Yates, F. A new method of arranging variety trials involving a large number of varieties. Jour. Agr. Sci. [England] 26: 424–455. 1936.

[3] Goulden, C. H. Methods of statistical analysis. Burgess Pub. Co., Minneapolis. (Mimeographed.) 208 pp. (rev.). 1936.

[4] Goulden, C. H. Efficiency in field trials of pseudo-factorial and incomplete randomized block methods. Canadian Jour. Res. (c), 15: 231–241. 1937.

QUASI-FACTORIAL ARRANGEMENT

15	13	16	14	4	2	3	1	6	5	7	8	9	11	10	12
10	14	2	6	3	7	11	15	4	12	8	16	1	13	9	5
5	8	6	7	15	16	13	14	9	10	12	11	3	2	4	1
1	5	9	13	14	2	6	10	3	7	15	11	8	4	12	16

RANDOMIZED-BLOCK ARRANGEMENT

2	10	13	14	3	16	14	6	9	7	5	11	8	12	15	1
13	7	9	4	2	5	10	15	6	14	3	1	16	12	11	8
4	9	1	12	3	2	13	8	6	7	11	15	14	5	16	10
2	5	10	3	14	16	15	7	13	11	4	8	1	6	9	12

FIG. 1. Diagrams of a two-dimensional quasi-factorial arrangement of plots and of a randomized-block arrangement for 16 hypothetical treatments or isolates.

pathogenicity of a large number of isolates of a particular pathogen is to be determined. In such experiments the seedling stand counts are usually made about 3 weeks after planting and before plant competition has become operative.

The writer, therefore, attempted to obtain definite information on the relative efficiency of 2-dimensional quasi-factorial experiments as compared with randomized-block tests in the field and greenhouse, where the emphasis is on seedling stands.

METHODS AND MATERIALS

Data were obtained from uniformity trials made with sugar beets in 1937 and 1938 in the field at Waseca, Minnesota. The land had been uniformly cropped in previous years. The seed was sown in 20-inch rows at about 20 pounds per acre. The experiments consisted of 36 plots in each of 4 replications, the plots being 4 rows wide and 30 feet long. Seedling counts were made, about 3 weeks after planting, in 6 four-foot lengths of row from the middle 2 rows of each plot.

Data already available[5] from 2 uniformity trials with sugar beets on 2 types of plant tables in the greenhouse were used for the present study also, with the following exceptions. In this study the seedling counts in the outside rows of pots surrounding each test were discarded, and the analyses are based on the stands in the remaining pots. This was done to eliminate the influence of heat from vertical coils of heating pipes situated along one side and at one end of each plant table.

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[5] LeClerg, E. L. Factors affecting experimental error in greenhouse pot tests with sugar beets. Phytopath. 25: 1019–1025. 1935.

Since the data considered were obtained from uniformity trials (*i.e.*, the plots and pots were sown to the same variety of sugar beets and at comparable rates), it is possible, by redistribution of assumed treatments, to analyze the data both as randomized-block and two-dimensional quasi-factorial experiments.

The data were analyzed by the analysis-of-variance method.[6]

EXPERIMENTAL RESULTS

Field Experiments

The analyses of variance of the seedling stand data were made on the assumption of 36, 25, 16, 9, and 4 hypothetical treatments in order to test the relative efficiency of quasi-factorial and randomized-block arrangements with experiments of different sizes.

The error variances (Table 1) of the quasi-factorial design are less than those of the randomized-block design for all of the 5 different sizes of experiments tested in both years.

TABLE 1.—*Increase or decrease in efficiency of the two-dimensional quasi-factorial design as compared with a randomized-block arrangement of equal size in field uniformity experiments dealing with damping-off of sugar beets*

Number of treatments	Error variance for		Increase or decrease in efficiency
	Quasi-factorial	Randomized-block	
Data for 1937			
			Per cent
36	2298.91	4106.11	+39
25	2164.39	2934.54	+ 2
16	2262.54	3273.02	+ 3
9	3213.86	3768.10	−22
4	3779.19	3945.56	−37
Data for 1938			
			Per cent
36	1142.84	1479.07	+ 1
25	1126.14	1484.11	− 1
16	1300.05	1820.73	0
9	1375.89	1754.39	−15
4	1060.19	1645.54	− 7

After correction of the error variances of the quasi-factorial arrangements for different sizes of experiments by their respective efficiency factors, it appears that, with 36 treatments (6 treatments in a block), this plot design was 39 per cent more efficient in 1937 and only 1 per cent more efficient in 1938 than a randomized-block test of the same size. When considering 25 treatments (5 treatments in a block), there was a gain of 2 per cent in 1937, whereas a loss of 1 per cent resulted in 1938. With 16 treatments, the quasi-factorial was 3 per cent more efficient in 1937 and was equal in efficiency to the randomized-block arrangement in 1938. Losses in efficiency of 22 and 15 per cent occurred with 9 treatments for the 2 years, respectively. Considering 4 treatments, a loss in efficiency of 37 per cent resulted in 1937 and 7 per cent in 1938.

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[6] Fisher, R. A. Statistical methods for research workers. Ed. 4, rev. and enl. 307 pp. Oliver and Boyd, Edinburgh and London. 1932.

Thus, with a large number of treatments under field conditions, the quasi-factorial arrangement of plots may be considerably more efficient than a randomized-block design in some seasons. With 25 or fewer treatments, a slight gain in efficiency may result, though a loss is more likely.

Greenhouse Tests

The analyses of variance of the stand counts for each of the pots were made on the basis of 25 hypothetical treatments on a board-wall bench and 36 hypothetical treatments on a raised-concrete bed. Each experiment consisted of 8 replications.

From the data in table 2 it is apparent that the quasi-factorial design is markedly less efficient than the randomized-block arrangement. The former was 16 per cent less efficient on the board-wall bench and 20 per cent less efficient on the raised-concrete bed. This is not surprising since the variability between pots in the greenhouse is not nearly so great as that between plots in the field.

TABLE 2.—*Loss in efficiency of the two-dimensional quasi-factorial design as compared with a randomized-block arrangement of equal size in greenhouse uniformity experiments dealing with damping off of sugar beets on two types of plant tables*

Number of treatments	Error variance for		Increase or decrease in efficiency
	Quasi-factorial	Randomized-block	
Board-wall bench			
25	190.89	212.80	*Per cent* -16
Raised-concrete bed			
36	151.46	155.05	*Per cent* -20

SUMMARY

The quasi-factorial design of plot arrangements was more efficient than a randomized-block test in some seasons for damping-off tests with sugar beets, with 36 treatments, in the field. With 25 or fewer treatments, the quasi-factorial design was less efficient in most comparisons, although it was more efficient in a few.

In greenhouse experiments, made on two types of plant beds, marked losses in efficiency resulted with quasi-factorial design as compared with a randomized-block arrangement.

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UNIVERSITY, LA.

PHYTOPATHOLOGICAL NOTES

A Method for Testing the Toxicity of Volatile Compounds.[1]—For testing the toxicity of volatile materials in the vapor phase, several methods have been used.[2, 3, 4] The technique reported herewith is an improvement of one of these[2] and has an advantage over the others, in that a minimum of special glassware is needed.

The containers are ordinary Petri dishes sealed with a double layer of grade "G" parafilm[5] or one-pint, glass-top fruit jars sealed by the usual rubber gaskets and wire clamps. The first of these may be used where a small volume of the solution is to be tested, the second where relatively large amounts are needed. The fungus culture is placed on an agar film deposited upon the inside of the cover of either Petri dish or fruit jar. It is essential to have the agar films uniform in diameter and thickness in order to avoid a differential in the rate of absorption of the volatile material to be tested which is placed in a solution of the desired concentration in the bottom of the container. The film is, therefore, poured in a sterile chamber where uniform discs can be cut and transferred to the containers.

The transfer chamber (Fig. 1, A) has an inclined glass window extending to within 10 inches of the bottom. In this 10-inch space is a door, hinged at the bottom, containing two 5-inch holes for hands and arms, and provided with sliding panels to close the openings when not in use. Compressed air is first passed through a 2-liter flask filled with sterile cotton (Fig. 1, A), then introduced into the chamber through a rubber tube to the end of which is attached a perforated glass pipe. This current of sterile air tends to drive out through the arm holes air-borne organisms carried in on the surfaces of containers and implements.

The agar film is made by pouring 400 cc. of melted agar onto a specially constructed plate within the chamber. For building this plate, heat-resisting plate glass, trade marked "Herculite," is secured from the Pittsburgh Plate Glass Co., Milwaukee, Wisconsin. The following dimensions

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[1] This work was supported in part by a grant from the Wisconsin Alumni Research Foundation.

[2] Walker, J. C., S. Morell, and H. H. Foster. Toxicity of mustard oils and related sulfur compounds to certain fungi. Amer. Jour. Bot. 24: 536–541. 1937.

[3] Oserkowsky, J. Fungicidal effect on *Sclerotium rolfsii* of some compounds in aqueous solution and in the gaseous state. Phytopath. 24: 815–819. 1934.

[4] Tomkins, R. G. The action of certain volatile substances and gases on the growth of mould fungi. Proc. Roy. Soc., Ser. B, 111: 210–226. 1932.

[5] Terry, M. C. To keep culture-media from drying out. Science (n.s.) 85: 319–320 1937.

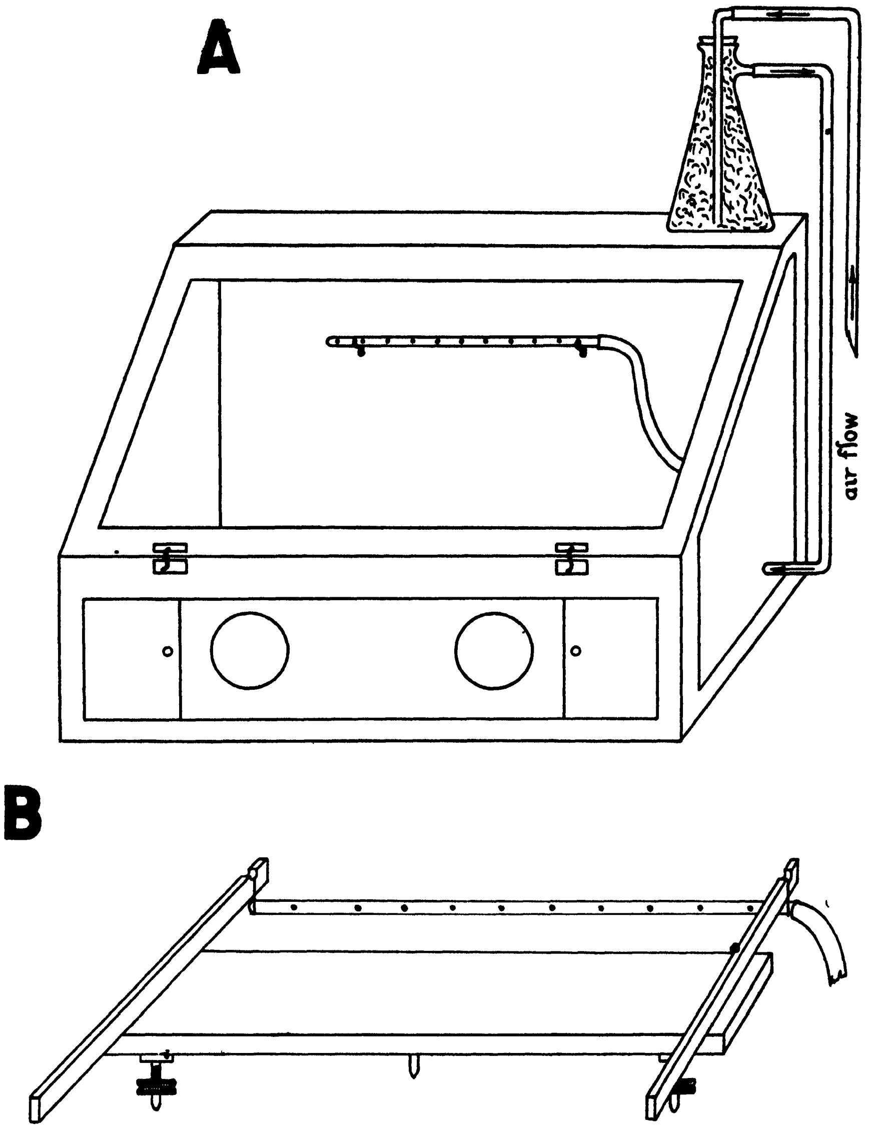

FIG. 1. A. Transfer chamber, illustrating filter flask and position of perforated glass tube when plate support is not in use. Scale: ⅛ in. = 1 in. B. Plate support showing position of perforated glass tube. Scale: ¼ in. = 1 in.

are required: 1 piece, 20 × 14 × ¼ in.; 2 pieces, 20 × ¾ × ¼ in.; 2 pieces, 12½ × ¾ × ¼ in. The narrow strips are cemented to the larger piece so as to form a shallow dish 18½ × 12½ × ¼ in. (inside dimensions), which is mounted on a small stand supported by 2 adjustable screws and a peg similar to those used on the bottom of an analytical balance (Fig. 1, B).

Before use the plate is leveled with the aid of a 6-inch carpenter's level. After the plate is set up and balanced, 4 small pieces of ordinary window glass (¾ × ¾ × ⅛ in.) are placed on the corners of the dish to support another sheet of window glass (21 × 15 × ⅛ in.) which serves as a cover. The plate and cover supports are sterilized by pouring over them a B–K solution (3.5 per cent sodium hypochlorite in water) diluted 1–5 with water. Next, the cover is wiped with the same liquid and put in place. The inside of the compartment is then sprayed with the disinfectant and, after a 10-minute period, the air turned on. Before entering the chamber the hands and arms are sponged with the solution. Most of the B–K is removed from the plate by a piece of sterile absorbent cotton. Alcohol is added and flamed and any moisture that remains is evaporated with a Bunsen burner. The agar is now poured on the plate and allowed to solidify. The perforated glass tube (Fig. 1, A) is then attached to the back of the stand and the sterile air directed beneath the plate, facilitating more rapid cooling of the agar. From the film, 2-inch discs are cut by means of a "biscuit-cutter" similar to, but much larger than, the one described by Keitt[6] and transferred to the containers with a sterile spatula.—DEAN E. PRYOR and J. C. WALKER, Department of Plant Pathology, University of Wisconsin, Madison, Wis.

Susceptibility of Seedlings of Ribes punctatum, an Andine Currant, to Cronartium ribicola.—Very little work has been done with regard to a study of the susceptibility of South American species of *Ribes* to *Cronartium ribicola* Fischer. So far as known the susceptibility of one of these, *Ribes punctatum* Ruiz and Pavon (Janczewski, E. de, Monographie des groseilliers, *Ribes* L. in Mém. Soc. Phys. et Hist. Nat., Genève, **35**: fasc. 3. Sg. V, Parilla; Sect. III, Euparilla. 1907), a dioecious Andine currant, which has little economic or ornamental value in the northern hemisphere, has not been investigated. In January, 1938, seed of *R. punctatum* were made available to the writer for propagation and testing by C. R. Quick, Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. This tender evergreen species grows on both slopes ot the Andes (Chile and Argentina) at approximately the latitude of Valparaiso, and probably is not at all hardy in the northeastern part of the United States.

The seed of *Ribes punctatum* germinated readily and 18 seedlings were potted. Later, in 1938, all of these seedlings, the sex of which was not known, became naturally infected with *Cronartium ribicola,* in a pathological greenhouse at the Marsh Botanical Garden, Yale University, New Haven, Connecticut. This infection occurred during the inoculation experiments on the second generation seedlings of the blister-rust-immune Viking (syn: Rød Hollandsk Druerips) red currant, and resulted from free urediospores produced on the susceptible *Ribes* "checks," the prickly gooseberry (*R. cynosbati* L.) and skunk currant (*R. glandulosum* Grauer). The leaves of *R. punctatum* were so readily susceptible to rust that the heavily infected ones became brown and shriveled. Abundant uredia and a trace of telia were present by the end of June. The rust continued to spread to new leaves, and in January, 1939, a collection was made showing mostly abundant, long, robust telia that had formed during the autumn and winter. Because of the very evident high receptivity of the leaves of seedlings of this South American currant to air-borne urediospores in the greenhouse, formal inoculation tests with *C. ribicola* were not performed.

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[6] Keitt, G. W. Simple technique for isolating single-spore strains of certain types of fungi. Phytopath. 5: 266–269. 1915.

In the autumn of 1937 the writer observed vigorous plants of *Ribes punctatum* with sizable leaves growing under greenhouse conditions at the University of California, Berkeley, California. If blister rust susceptibility of matured plants of *R. punctatum* is not significantly different from that of seedlings reported in this note, the species should be useful as a source of telia inoculum for infection experiments with five-needle pines carried on in the greenhouse during the late autumn and winter. At this time North American and European *Ribes* have dropped their leaves and fresh telia on new leaves are not available.—GLENN G. HAHN, Division of Forest Pathology, Bureau of Plant Industry, U. S. Dept. of Agriculture in cooperation with Osborn Botanical Laboratory, Yale University, New Haven, Connecticut.

Early Blackhull Resists Stem Rust in Texas.—Early Blackhull winter wheat, a variety that has recently come into prominence in the Southern Great Plains, has been found to have a high degree of early resistance to stem rust (*Puccinia graminis tritici* Eriks. and Henn.) in tests conducted on several of the experiment stations in Texas. At College Station, Texas, it has, for the past 3 years, consistently remained almost free from stem rust for about 2 weeks after other varieties that head at the same time have become heavily infected. The same observation was made by the writer in 1938 on the Texas substations at Angleton on the Gulf Coastal Prairie, at Beeville on the Rio Grande Plain, and at Temple in the Black Lands of Central Texas. I. M. Atkins has reported to the writer a like performance of this variety on the Denton substation in northern Texas.

About a week or 10 days before ripening, Early Blackhull usually develops a light to fairly abundant infection characterized by small, distinctly isolated pustules of stem rust, which, due to their late appearance, do not usually cause much injury. This performance has been consistent at College Station in the known presence of the following physiologic races of *Puccinia graminis tritici* 1, 11, 17, 21, 24, 36, 38, 49, 50, and 56. Obviously, the variety either possesses early resistance to several races of stem rust or the rust organism requires a long incubation period on this host.

In crosses between Early Blackhull and varieties carrying the Hope type of mature-plant resistance to stem rust, an occasional F_2 plant has been observed to remain entirely rust-free, both early and late in the season, under conditions where both parents have developed considerable infection and where Gasta checks have succumbed before heading. It is assumed that these rust-free segregates carry a factor, or factors, for early resistance derived from Early Blackhull combined with the factor for mature plant resistance derived from Hope.

Although Early Blackhull is reported to be of poor milling quality, its early maturity, resistance to shattering, and early resistance to stem rust apparently make this variety of considerable value as a parent in the breeding of winter wheats for the Southern Great Plains, especially the extreme southern part of that area, where stem rust is able to develop during the winter and early spring months.—E. S. MCFADDEN, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Texas Agricultural Experiment Station.

Rust on the California Native Pruni.—*Tranzschelia pruni-spinosae* (Pers.) Diet. has, to the knowledge of the writers, never been reported on

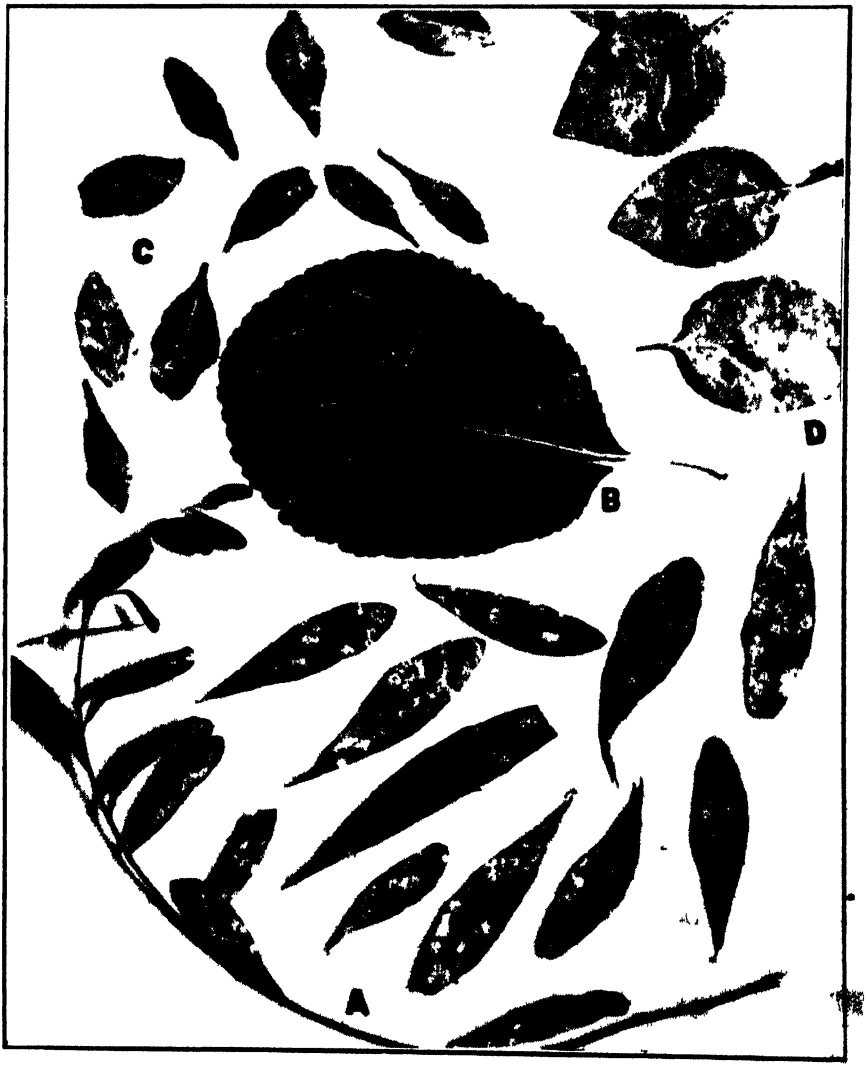

FIG. 1. Rust sori on the leaves of the California species of native *Prunus*: A, *P. fasciculata*; B, *P. subcordata*; C, *P. andersonii*; D, *P. fremontii*.

the wild species of *Prunus* in California, and no rust has been observed on these plants when grown in their natural habitat, one approaching near desert conditions with most of the species. The following native species have been grown under cultivation in a mixed planting with other species of *Prunus* and *Amygdalus: Prunus andersonii, P. demissa, P. emarginata, P. fasciculata, P. fremontii, P. ilicifolia, P. ilicifolia* var. *integrifolia,* and *P. subcordata.*

In the mixed planting rust has been observed frequently on the susceptible horticultural varieties of *Prunus armeniaca, P. domestica,* and *Amygdalus persica.* This provided abundant inoculum to infect the native species. All indigenous species listed were susceptible to rust, except *P. demissa* and *P. ilicifolia.* Two of the desert forms, *P. andersonii* and *P. fasciculata* (Fig. 1, A and C), developed a few brownish uredia, scattered over the leaf surface, with little or no necrosis of tissue surrounding the sori. The urediospores were abundant, and teliospores (discolor type) have been observed on *P. fasciculata. Prunus fremontii,* another desert species. developed necrotic spots 2–5 mm. in diameter (Fig. 1, D), with a few brownish (sometimes whitish), imperfectly formed uredia. The urediospores were few. *Prunus subcordata* is very susceptible to rust and develops large uredia widely distributed over the leaf surface with abundant urediospores. No teliospores were observed. *Prunus emarginata* was infected after artificial inoculation in the open with rust spores from almond. The rust, where teliospores developed on these species of *Prunus,* was of the *discolor* type, as reported by Dunegan.[1] The *typica* type of rust has not been observed by us in California.—CLAYTON O. SMITH AND L. C. COCHRAN, University of California, Citrus Experiment Station, Riverside, California.

The Number of Spores in a Pycnidium of Septoria apii.—Recently 280 samples of celery seed obtained from New York State growers were examined by A. G. Newhall, who found that 142 samples, or about 50 per cent, contained seeds bearing pycnidia of *Septoria.*

The ability of the *Septoria* fungus to bridge the gap from seedcoat to young seedling depends upon its viability, suitable environmental conditions in the seedbed, and to a considerable extent upon the amount of initial inoculum or the "inoculum potential." Considerable is known about the first 2 conditions, but the number of spores that can be liberated at one time by a single pycnidium seems not to have been ascertained. After a number of preliminary attempts, the writer finally adopted the following method of counting all the spores from a single pycnidium.

Under a low-power binocular microscope a pycnidium from a young lesion was dissected out and placed in a drop of tap water on a clean slide. A pair of needles were used to break open the pycnidium and to separate any spore clumps, thus facilitating a fairly uniform distribution of spores throughout the drop.

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[1] Dunegan, J. C. The rust of stone fruits. Phytopath. 28: 411–427. 1938.

This drop of spore suspension was then allowed to dry. A drop of glycerine and a coverglass were added and counting was done under low power. All spores were counted, and accuracy was facilitated by the substitution of a "counting field" in the ocular of the microscope for the ordinary ocular micrometer. This was a round glass disc lightly ruled off with a glass cutter into 16 small squares. Care was taken to have at least the outer lines of this figure parallel so that no spores would be overlooked or counted twice on moving the slide from one "field" to another. It required nearly an hour to count the spores from 1 pycnidium. Two people, making counts of the same mount, were able to check within less than 0.9 per cent. The results of 9 counts are as follows:

Pycnidium	1	2	3	4	5	6	7	8	9	Ave.
No. of spores	3206	5317	2894	4140	2304	5493	1448	3314	4960	3675

This indicates then the potential inoculum released from 1 pycnidium of *Septoria apii graveolentis.* Although these pycnidia came from leaves, it is assumed that pycnidia borne on seeds would contain a like number of spores. In addition to the number of spores in one fruiting body, the number of pycnidia in a single lesion also are important in determining the inoculum potential. Therefore, counts of the number of pycnidia per lesion were made on a number of severely infected celery plants approximately 10 inches tall and are given below

Lesion	1	2	3	4	5	6	7	8	9	10	11	12	13	Ave.
No. pycnidia	23	20	25	12	91	39	14	87	7	38	30	129	219	56

It would seem from these figures that if only 10 primary lesions occurred in a seedbed there would be possibly 1½ million spores available as secondary inoculum long before the plants are transplanted to the field. This all points to the importance of Septoria-free seed and explains in part at least the universal success that has attended the practice of thoroughly dusting or spraying celery plants several times while still small in the seedbeds.—K. H. LIN, Cornell University, Ithaca, New York.

Further Data on Breeding Mosaic-escaping Raspberries.[1]—Data published previously[2, 3] showed that the Lloyd George red raspberry variety is resistant, possibly immune, to the mosaic vector *Amphorophora rubi* Kalt. but is heterozygous for resistance. In several small populations produced by inbreeding the variety Lloyd George and crossing it with susceptible varieties, a relatively small number of the seedlings were resistant. Several varieties and seedlings proved to be somewhat resistant. Other data indicated that susceptible varieties may be homozygous, but the numbers of individuals tested were too small to permit definite conclusions. Subsequent greenhouse experiments have added materially to our conception of the genetics of resistance. The results (Table 1) represent the relative survival and reproduction of *A. rubi* upon 5 potted "sucker" plants of each inbred or hybrid seedling.

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[1] Published as Scientific Paper No. 401, College of Agriculture and Experiment Station, State College of Washington.

[2] Schwartze, C. D., and Glenn A. Huber, Science 86: 158–159, 1937.

[3] Huber, Glenn A., and C. D. Schwartze, Jour. Agr. Res. 57: 623–633, 1938.

TABLE 1.—*Resistance to Amphorophora rubi in hybrid and inbred red raspberry populations*

Parentage	Number of seedlings tested	Resistant	Somewhat resistant	Susceptible
		Per cent	*Per cent*	*Per cent*
Cuthbert (susceptible) × self	8			100.0
Lloyd George (resistant) × self	50	72.0	12.0	16.0
WSC 22 (Lloyd George selfed, somewhat resistant) × Lloyd George	31	64.5	6.5	29.0
WSC 43 (Lloyd George × Cuthbert, resistant) × Lloyd George	45	80.0	2.2	17.8

All of the individuals listed in table 1 differ from those previously tested. The 8 Cuthbert selfs raise the total tested to 14 and furnish further evidence that susceptible types are homozygous. The data for Lloyd George selfed differ from those previously published in that a much higher percentage of the seedlings show resistance.

Aphid counts in the field were in close agreement with the results of the greenhouse experiments. The number of Lloyd George selfs tested now totals 66, of which 40 are resistant, 8 somewhat resistant, and 18 susceptible.

The 2 hybrid populations show that relatively high percentages of resistant seedlings can be produced by crossing heterozygous parents. It is strongly indicated that Lloyd George carries 2 or more factors for resistance and that resistance is dominant to susceptibility. WSC 22 × Lloyd George produced a higher percentage of resistant hybrids than did crosses between Lloyd George and susceptible parents.[3] It appears likely that the somewhat resistant type carries at least 1 genetic factor for resistance, but further study is necessary. WSC 43 carries more resistance factors than WSC 22, as shown by the higher percentage of resistant individuals in the cross WSC 43 × Lloyd George than in WSC 22 × Lloyd George.

There are now in the breeding plots 111 hybrids, inbred seedlings, and named varieties on which *Amphorophora rubi* does not feed. Most of these must be heterozygous for resistance because of their parentage. Further inbreeding and hybridizing, now in progress, should produce homozygous resistant individuals that will transmit resistance to 100 per cent of their offspring when crossed with susceptible parents.—C. D. SCHWARTZE AND GLENN A. HUBER, Washington Agricultural Experiment Station and Western Washington Experiment Station.

All these types have been observed in Idaho prune orchards, but the gum spot or drought spot has been most prominent because it often accompanies one or more of the other symptoms. Several years' observations in Idaho have shown the following:

1. Prunes affected by drought spot are unusually firm of flesh and poor in quality. No improvement of fruit in storage is apparent.
2. The firmness of flesh is attributable in large part to dead, corky tissue areas and a pronounced sticking to the pit.
3. Internal discoloration, particularly near the pit, is common.
4. The presence of these hard-flesh, unpalatable prunes, especially in the dried product, constitutes a serious and neglected problem.
5. Drought spot is more severe in years of inadequate irrigation water, especially on light, porous soils.

Tests with boron applications to prune trees in Idaho have been conducted since 1936, but no conclusive data on recovery are yet available. Reports from certain other areas where similar tests are in progress indicate, in general, unsatisfactory results.

Preliminary data, however, on analyses of drought spot and healthy fruit have shown a definite relation between the disease and low boron content of fruit tissue and pits. Furthermore, drought spot of prunes has been especially severe in one section of Idaho where it already has been shown[2] that, for alfalfa, the soil is deficient in boron. The low boron content of apple tissue produced on [illegible] of this area was correlated with high incidence of drought spot and corky core.

More complete data on drought spot of prune are expected to be available in the near future, but a brief preliminary note at present seems to be warranted by the circumstances.—EARLE C. BLODGETT AND WILLIAM E. COLWELL, Departments of Plant Pathology and Agronomy, University of Idaho, Moscow, Idaho.

Botrytis Blight of Antirrhinum Related to Trichome Disposition.[1]—About 25 per cent of the *Antirrhinum majus* plants, varieties After Glow and Cheviot Maid Supreme, in a greenhouse near Albany, Oregon, were broken over in November, 1938, by a Botrytis infection (Fig. 1). The *Botrytis* proved to be a cinereal form of the type that produces long dark-brown conidiophores on the suscept. The grower, when watering the snapdragon plants, was careful not to wet the foliage; but, in November, he was forced to spray the foliage with an insecticide against thrips. The Botrytis attack developed shortly after wetting the foliage with the insecticide. The plants broke over consistently at a point about 2 feet from the ground line. An examination of several hundred of these plants showed that the foci

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[2] Colwell, W. E., and G. O. Baker. Studies of boron deficiencies in Idaho soils. Jour. Amer. Soc. Agron. (Accepted for publication.)

[1] Published as Technical Paper No. 305 with the approval of the Director of the Oregon Agricultural Experiment Station. Contribution from the Department of Botany and U. S. Department of Agriculture, Bureau of Plant Industry, Fruit and Vegetable Crops and Diseases, cooperating.

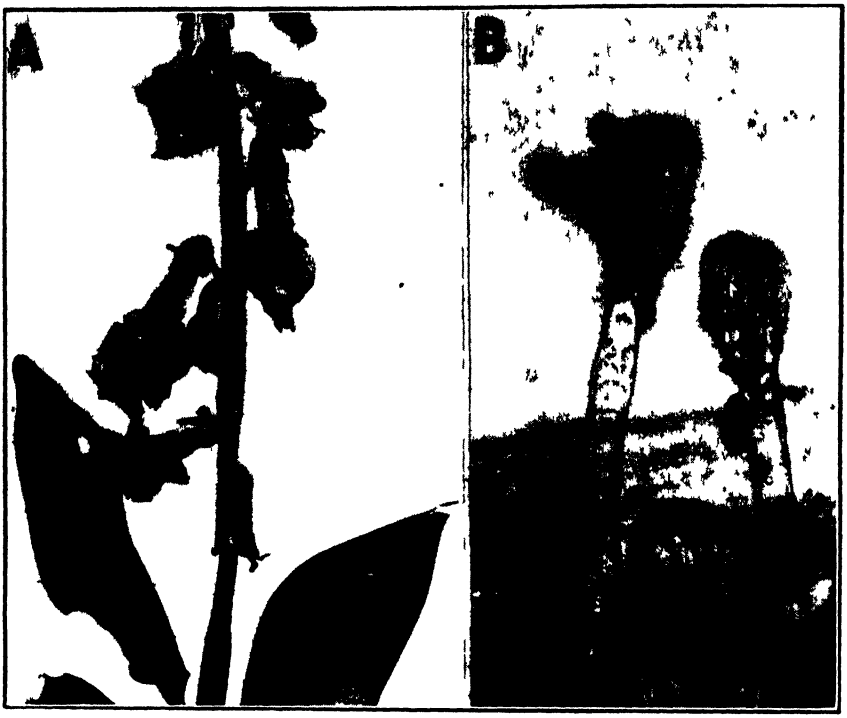

FIG. 1. A. Habit of *Antirrhinum* stem showing location of *Botrytis* attack. B. Group of trichomes. A *Botrytis conidium* is sending a germ tube into the head of the longer trichome.

of primary infections correspond with the locations where the surfaces of the stems change from glabrous to hirsute. The glandular trichomes in affected stem areas were full of *Botrytis* mycelium; moreover, infection through trichomes was observed. It is not claimed that the infection of snapdragon is always delimited by trichomes, but in this particular instance they served as effective infection courts either because they retained moisture or because of some inherent property that made them serve as suitable media for germination and infection.—FRANK P. MCWHORTER, Oregon Agricultural Experiment Station, Corvallis, Oreg.

Phytophthora Stem Rot of Viscaria.[1]—A stem rot of *Viscaria* (*Lychnis viscaria* L.), variety blue pearl, was observed by the writer at Lafayette, Indiana, in 1937. *Phytophthora cactorum* (L. and C.) Schroet. has been identified as the causal organism. The symptoms, etiology, and histology of the disease are reported here.

The disease is confined to the lower part of the stems and to the roots of the plants (Fig. 1, A). A water-soaked area on the young stem is the first symptom. Later the infected parts become dry, light brown, somewhat sunken, and partly disintegrated. Recently infected plants may wilt during the heat of the day and recover turgidity during the night. In later stages of the disease, the plants wilt permanently and die.

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[1] Contribution from the Department of Botany, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

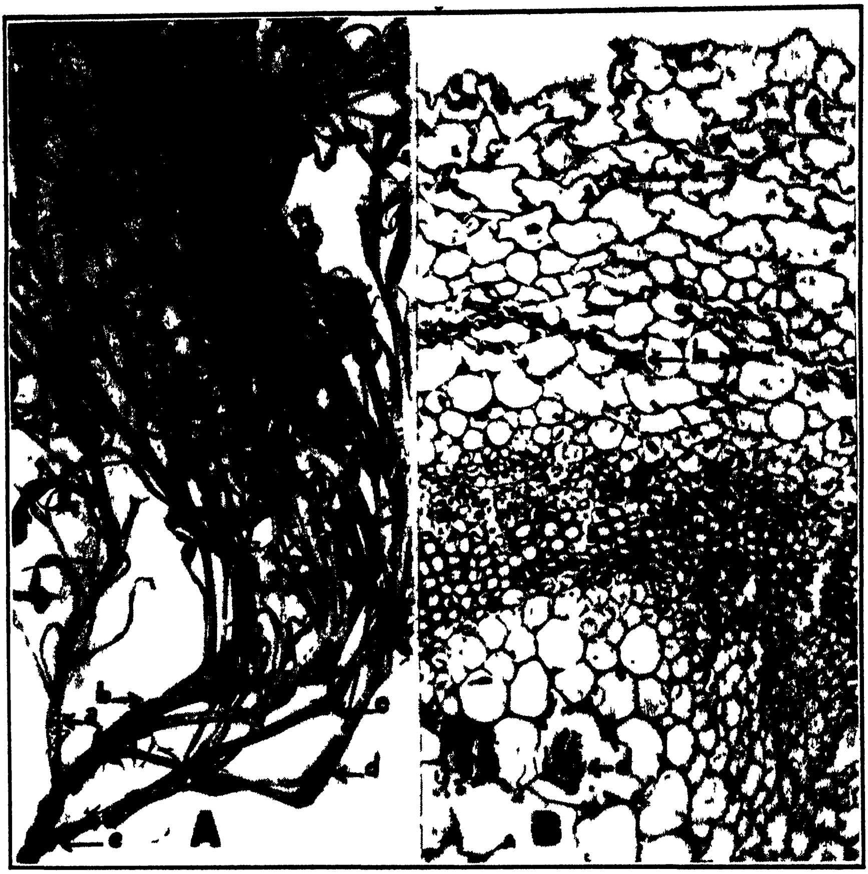

FIG. 1. A. Viscaria plant showing stem rot. The upper margins of decay are indicated (a–d). Stem (e) was dry, cracked, and partially disintegrated. × 0.6. B. Cross section of infected stem. Hyphae of *Phytophthora cactorum* in the cortex are indicated at (a), in the phloem at (b), in the xylem at (c), and in the pith at (d). Note haustorium-like branching of hypha in cell of the pith (d).

Pieces of young stems showing typical lesions of the disease were fixed in Rawlin's solution, infiltrated in paraffin, and sectioned by the usual method for histological examination. In sections stained in anilin blue in picric acid,[2] safranin, and orange G, the fungus, which stained blue, was clearly differentiated.

The mycelium of the fungus penetrates all of the tissues of the stem, is both inter- and intracellular, and is especially abundant in the tissues of the cortex, phloem, and pith (Fig. 1, B). Occasionally, mycelium within large cells of the pith branches profusely (Fig. 1, B, d). Abundant oospores were found in tissues of the cortex, endodermis, and stele. Host cells, exclusive of the xylem, in the infected area usually were partially collapsed.

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[2] Cartwright, K. St. G. A satisfactory method of staining fungal mycelium in wood sections. Ann. Bot. 43: 412–413. 1929.

Apparently pure cultures of *Phytophthora cactorum* were isolated from small pieces of infected stems that had been surface sterilized in 1/1000 mercuric chloride and planted on potato-dextrose agar.

The pathogenicity of the fungus was tested on 10 young *Viscaria* plants, 5–6 inches high and growing in pots in a greenhouse. The inoculations were made by placing a small piece of mycelium and agar, from a 3-day-old culture on potato-dextrose agar, on an unwounded stem an inch above the soil. Moist cotton was placed around the inoculations to prevent drying. Typical cankers resulted on 6 of the 10 plants, one week after inoculation. No infection occurred on 10 check plants treated as the inoculated plants, except that sterile potato-dextrose agar was used in place of fungus inoculum. *Phytophthora cactorum* was readily isolated from infected plants.

A culture of *Phytophthora cactorum* isolated from *Viscaria* was nonpathogenic when inoculated into the trunks of 2 21-year-old Grimes apple trees. However, typical collar-rot cankers developed from inoculations made on the trees with a culture of *P. cactorum* isolated from an apple-trunk canker. The culture of the fungus from *Viscaria* thus differed in pathogenicity on Grimes trees from numerous cultures of *P. cactorum* isolated from cankers on Grimes trunks and from apple fruits in Indiana.[3]

The *Phytophthora* isolated from *Viscaria* agrees in cultural and morphological characteristics with those established by Tucker[4] and Leonian[5] for *P. cactorum.* Cultures of the fungus grew well on malt extract agar and produced oogonia abundantly on oatmeal and pea agars. The cultures grew rapidly at 26–28° C. on potato-dextrose agar. Oogonia, formed after transfer of the fungus from pea broth to distilled water, averaged 26 μ in diameter. Antheridia, both in culture and within the host, were predominatingly paragynous. Sporangia were papillate.—R. C. BAINES, Purdue University Agricultural Experiment Station, Lafayette, Ind.

Anthracnose of Lippia.[1]—While making a survey of the commercial mint fields of northern Indiana we discovered plants of *Lippia lanceolata* Michx. heavily affected with leaf and stem lesions (Fig. 1, A) so similar to those of anthracnose of mint[2] (caused by *Sphaceloma menthae* Jenkins[3]) that we thought the causal organism might be the same. Microscopic examination disclosed a fungus referable to *Sphaceloma* and similar in habit (Fig. 1, A, B) to *S. menthae*, but with smaller spores (2–2.8 × 3.8–5.7 μ as

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[3] Baines, R. C. Phytophthora trunk canker of apple. (Abstract) Phytopath. 28: 2. 1938.

[4] Tucker, C. M. Taxonomy of the genus *Phytophthora* de Bary. Mo. Agr. Exp. Stat. Res. Bull. 153. 1931.

[5] Leonian, L. H. Identification of *Phytophthora* species. W. Va. Agr. Exp. Stat. Bull. 262. 1934.

[1] Contribution from the Department of Botany, Purdue University Agricultural Experiment Station, Lafayette, Indiana.

[2] Baines, R. C. Mint anthracnose. Phytopath. 28: 103–113. 1938.

[3] Jenkins, Anna E. New species of *Sphaceloma* on *Aralia* and *Mentha.* Jour. Wash. Acad. Sci. 27: 412–414. 1937.

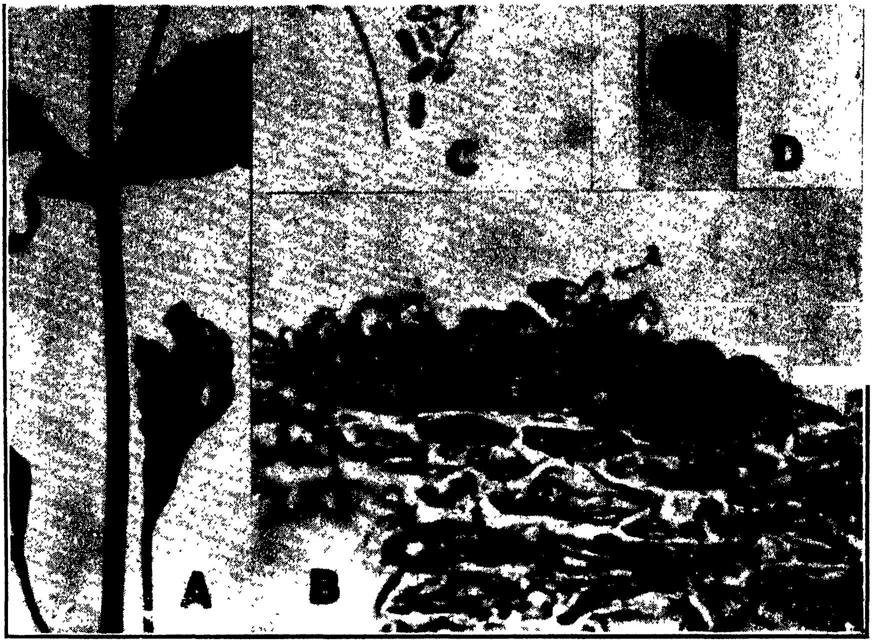

FIG. 1. A. Anthracnose lesions on stems of *Lippia lanceolata* are roundish, small, or attaining a diameter of ¼ in. They frequently coalesce to form large cankered areas. The lesions are slightly depressed and light colored, with a purplish-red margin. On leaves the disease first occurs as small reddish spots, which enlarge, become brown, and frequently the infected areas drop out. × 1. B. Cross section through a stem lesion, showing superficial stroma, and spores at (a). C. Conidia. Both × 870. D. Month-old colony of *Sphaceloma lippiae* on potato-dextrose agar. × 0.8.

against 2.5–4 × 3–8 μ). The fungus was isolated and, on inoculation with spores, produced the disease in *Lippia* but failed to infect *Mentha piperita* L. var. *vulgaris* Sole. Reisolation was successful. Moreover, plants of *Lippia* were not infected by inoculation with spores of *S. menthae*. The fungus thus differs from *S. menthae* both in morphology and parasitism and is considered a new species.

Sphaceloma lippiae, sp. nov.

Maculis caulicolis vel foliicolis, numerosis, sparsis vel dense aggregatis et plus minusve confluentibus, purpureo-vinaceis, in centro ochraceis, rotundis, ellipticis vel irregularibus, leniter depressis, 0.5–2.0 × 1.0–5.0 mm.; acervulis erumpente superficialibus, stromatibus pallide brunneis exilis; conidiis unicellularibus, oblongo-ellipticis, hyalinis, 2–2.8 × 3.8–5.7 μ. Hab. in foliis caulibusque vivis *Lippiae lanceolatae* in Indiana.

Lesions foliicolous or caulicolous, numerous, scattered or closely aggregated and commonly becoming confluent, surrounding host tissue wine-color, the sporiferous region buff, depressed due to collapse of underlying tissue, round, elliptical or irregular in shape, 0.5–2 × 1.0–5.0 mm.; acervuli subcuticular in origin but early becoming superficial; stroma thin and pale-brownish; conidia sessile and produced by budding of the cells of the stroma, unicellular, oblong-ellipsoid, hyaline, 2–2.8 × 3.8–5.7 μ. On living stems and leaves of

Lippia lanceolata Michx., North Liberty, Indiana, August 8, 1938, George B. Cummins and R. C. Baines. Type deposited in the Arthur Herbarium, Purdue University Agricultural Experiment Station.

Sphaceloma lippiae grows slowly on potato-dextrose agar and forms a brown to gray, heaped-up colony with a reddish margin (Fig. 1, D). The medium takes on a light wine color. Conidia obtained from cultures of the fungus are somewhat larger (3.4 × 8 μ) than those from lesions on the host. —R. C. BAINES AND GEORGE B. CUMMINS, Purdue University Agricultural Experiment Station, Lafayette, Ind.

Prevalence of Basisporium gallarum in Arrested Axillary Shoots and Secondary Ears of Maize.[1]—References to the infection of ears and shanks of maize by *Basisporium gallarum* Molliard are common in the literature, but no one has reported the infection of arrested axillary shoots or the more common occurrence of this fungus in secondary than in primary ears.

Basisporium infection of arrested axillary shoots was first noted in the autumn of 1937. A preliminary examination of 182 such shoots collected in different localities in Iowa showed that 77 (42.3 per cent) were infected with this fungus. Such a high percentage of infection is of especial interest because, in 1937, the percentage of infected ears was low, causing an estimated loss of only 1 per cent. In 1938, on the other hand, Basisporium infection was more general in Iowa. An extensive examination of arrested axillary shoots in the autumn gave the following results:

Source of material	Number of shoots examined	Number of infected shoots	Percentage of infected shoots
Open-pollinated dent corn (eight localities)	474	382	80.59
Inbred lines, Ames	551	361	65.51
Single crosses, Ames	531	411	77.40

The susceptibility of these tissues is very evident, with average infection ranging from 65.51 to 80.59 per cent in the material examined.

It also was observed that the secondary ears were more frequently infected with *Basisporium* than were the primary. Of 604 paired primary and secondary ears gathered in 3 localities, 307 of the secondary and 13 of the primary ears were infected.

A relation was noted between degree of development of secondary ears and percentage of infection. Ninety-seven secondary ears, the weight of each exceeding 7/10 that of the respective primary ear, included only five infected ears. In contrast, 273 secondary ears, none of which weighed more than ¼ as much as the respective primary ear, included 191 infected ears. Susceptibility to infection appears to be favored by poor development in secondary ears.

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[1] Journal Paper No. J-625 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 94.

The common occurrence of *Basisporium* infection of arrested axillary shoots and poorly developed secondary ears indicates that these structures are the most susceptible tissues of the corn plant. These tissues were most heavily infected in 1938, a year of abundant *Basisporium* infection, but better evidence of their susceptibility was obtained in 1937, when the reported infection was low. It is obvious that such extensive infection of arrested axillary shoots and secondary ears insures abundant inoculum adjacent to a large percentage of the primary ears.—J. H. Standen, Iowa Agricultural Experiment Station, Ames, Iowa.

REVIEW

Cochran, W. G. Some difficulties in the statistical analysis of replicated experiments. The Empire Jour. of Exp. Agr. 1: 157–175. 1938.

Salmon, S. C. Generalized standard errors for evaluating bunt experiments with wheat. Jour. Amer. Soc. Agron. 30: 647–663. 1938.

Clark, A., and W. H. Leonard. The analysis of variance with special reference to data expressed as percentages. Jour. Amer. Soc. Agron. 31: 55–66. 1939.

Attention is called to these papers, particularly the first, as ones that would well repay study by pathologists who are working with quantitative data involving mortality or morbidity percentages or other disease-intensity ratings. They deal with the practical consequences of the fact that such percentages between 30 and 70 are much more variable than those below 10 or above 90. Zero and 100 act as limiting values, and the variation of percentages near them cannot be so great as that of percentages nearer the middle of the scale. In all three papers plant-disease data are discussed. They direct attention to mistakes in conclusions that may arise as a result of uneven variation, either in the analysis of variance or in any other procedure by which a generalized error is computed for an entire experiment.

Salmon shows in some particularly interesting graphs the variation actually found in different parts of the percentage scale in a large body of bunt-infection data. He proposes for computation of the variance applying to any particular percentage, the well-known formula $\frac{100\,pq}{n}$, multiplied by a factor to be determined from the data that are being handled and related to the heterogeneity of the particular experiment. His graphs give indication of even greater difference between the variation at the ends and that near the middle of the percentage scale than would be corrected by this method.

A simple procedure that meets the difficulty in some cases, is to take as basis for a generalized error, only those categories that show approximately equal variation. For example, untreated units or treatments that have obviously failed may be excluded from the analysis when testing significance of differences between promising treatments. It is sometimes desirable to make separate error determinations for categories or groups of categories that appear to have materially different variances, as, for example, inoculated and non-inoculated units. In routine analysis on yields of English field crops, Cochran finds it apparently successful to omit from the main analysis treatments that yield consistently more than double or less than half the main group of treatments. While he recognizes that various kinds of data may be found on inspection to show uneven variance and need special consideration, the types for which there is *a priori* basis for special handling are limited. He points out that in some experiments the percentages that need to be included in an analysis lie between 30 and 70, within which limits the variation is not sufficiently correlated with the mean to necessitate special treatment. He further regards as usually needing no extraordinary treatment, percentages based on fractions whose numerators exceed 100. For percentages requiring special treatment because they are percentages, he favors transformation to a scale in which the variances of the different treatments are approximately equal. The function of transformation he defines as the conversion of skew distributions into distributions that are approximately normal with the same variance. The procedure is to transform each observation; conduct analyses with the transformed values; and then in some cases reconvert the means of the transformations back into the original form. The transformation that he recommends for general use in preparing mortality and morbidity percentages for analysis of variance is the inverse sine or equivalent angle, which had been privately proposed by Fisher some years earlier, and for which tables with discussions are available.[1] One kind of empirical test that can be made of applicability of a transformation to a particular type of data is to compare the skewness of the original values with that of the transformed values for several different samples; a simple test for skewness is to compare the number of items above the mean with the number below it.

Clark and Leonard indicate that the inverse sine transformation is applicable only to percentages based on discrete data, as the percentage of individuals. They state that percentages based on continuous data, as for example weights of material, should not as a rule be transformed for the purpose of equalizing variance; but they recognize that each problem involving percentage data must be considered to determine whether transformation is called for. In a specific case involving percentages of area covered by competing organisms $\log \frac{1}{1-p}$ is proposed as a transformation. They point out that percentages computed on an identical base have the same distribution as the numerators of the fractions from which they are derived, and require no special treatment, unless the distribution of the numerators themselves was such as to require it. Tests are described for determining whether data are so heterogeneous as to need transformations or eliminations.

For some kinds of values used by pathologists, others of the tranformations suggested by Cochran will undoubtedly be found useful. Among these are logarithms, regularly used in computing the geometric mean (the mean that should be used in averaging percentages obtained by expressing the result of one treatment as a percentage of another with which it is being compared). For small-number distributions (Poisson), such for example, as might be encountered in reporting the number of infections per leaf when disease is not very prevalent, or the colonies of a particular fungus caught on exposed Petri dishes, he approves the square root transformation, and if values are below 10, the similar $\sqrt{x+\frac{1}{2}}$. If the data extend over a very wide range, as such material often does, he suggests the tranformation $\log(x+1)$, as behaving like the square root for numbers up to 10, and thereafter like the direct log.

The papers under review do not discuss the effect of uneven variance on averages. Uneven variance not only makes dangerous the application of a generalized error; it also may introduce concealed and sometimes undesirable weights into averaging. For example, one may need to combine the results of duplicate experiments run at different places or in different years, in one of which infection was light with percentages generally around 10, and in the other heavier with percentages in the neighborhood of 40. A straight averaging of the percentages for the two experiments will give the second one the greater influence on the combined results because of the greater variation that will be found in the percentages at the higher level. In such a case the derivation of means from suitably transformed values, whether in an analysis of variance or otherwise, increases the likelihood of arranging the different treatments in correct order of effectiveness. But it must be kept in mind that an average derived by converting the mean of the transformed values back into the original scale not only differs from the simple arithmetic mean of the original values, but lacks one of its important qualities; it is not possible to estimate, from the mean reached through a transformation, the aggregate value for the population represented by the sample. The arithmetic mean of the raw data must, therefore, be used in averaging loss figures in samples in order to obtain an estimate of total loss in the population they represent, as also for estimating the magnitude or dollar value of the differences between treatments. For such purposes, the heavier weight that this may give the trials with heavier mortality is not particularly undesirable, since preventive treatments are used on many crops for their insurance value against heavy losses rather than for their value in cases of light or ordinary infection. Even for ranking treatments in order of effectiveness, heavier weight on the most severe trials is sometimes desirable, since there probably are diseases in which the treatments most effective under conditions of light infection will not be the most effective ones under more severe conditions.

Eliminations or transformations will be helpful in many cases in the analysis of disease percentages or indices, but are often unnecessary and in any case must be used with discrimination. The analyst must define clearly the questions he is trying to answer.—CARL HARTLEY, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

[1] Fisher, R. A., and F. Yates. Statistical tables for biological, medical and agricultural research. Oliver and Boyd [Edinburgh]. 1938.

Bliss, C. I. The analysis of field experimental data expressed in percentages. With English summary. Institute of Plant Protection, Leningrad. Plant Protection, No. 12. 1937.

FACTORS AFFECTING THE DEVELOPMENT OF PUCCINIA CORONATA IN LOUISIANA[1]

IRVIN L. FORBES[2]

(Accepted for publication March 28, 1939)

INTRODUCTION

Crown rust of oats, *Puccinia coronata* Cda., is the limiting factor in the commercial production of oats in the southern United States. In Louisiana it reaches epidemic proportions every year, and only the more resistant varieties of the cultivated red oat group, *Avena byzantina* C. Koch., can be grown with any degree of success.

Species of *Rhamnus* that serve as alternate hosts for *Puccinia coronata* do not occur in Louisiana. It has been assumed, therefore, that the rust persists from year to year in the uredial stage. That it can develop well in the winter is indicated by the fact that it usually is quite prevalent at least from mid-winter until oats mature. It also has been assumed that urediospores can withstand the high temperatures of summer. It would be necessary for them to remain viable if the rust persists throughout the year in the South, because there are no green plants of oats or of susceptible grasses during the summer.

As very little precise information is available regarding the factors affecting the development of epidemics in Louisiana, it seemed desirable to investigate the problem. While other investigators have collected data regarding the development of epidemics, and have made experiments on the factors affecting spore viability and germination, there was relatively little information that would apply to conditions in Louisiana. As a basis for understanding the sequence of events in the development of epidemics, it seemed desirable to investigate the factors affecting the vitality and germination of urediospores, and the factors affecting the infection of host plants and subsequent development of rust. In addition, field observations were made in order to determine what happens in nature.

If the alternate host plays no part in the epidemiology of crown rust in the far South, the only feasible method of controlling the disease adequately would be the use of resistant varieties. For this reason tests of varietal susceptibility were made for 3 years and observations were made on the nature of resistance, but complete solution of so complex a problem has been impossible.

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[1] Presented to the faculty of the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Degree granted June, 1935.

[2] The writer wishes to acknowledge his indebtedness to the following people: Thanks are due Dr. C. W. Edgerton, Plant Pathologist of the Louisiana State University, for suggesting the problem and for encouragement during the course of the work. The writer is deeply grateful to Dr. E. C. Stakman, under whose direction this work was done, for valuable suggestions and criticisms during the course of the work and in the preparation of the manuscript. To Dr. Helen Hart, with whom some of the work was done, and who gave stimulating encouragement and much help in making the experiments and in the writing of the paper, my sincerest thanks are here acknowledged. Further thanks are here expressed to Mr. John Gray of the Louisiana Experiment Station for cooperating in the field varietal tests of oats to crown rust in Louisiana.

EXPERIMENTAL RESULTS

The studies reported here have dealt with the effects of temperature on viability of urediospores, percentage and rapidity of germination of urediospores, and development of rust after infection occurs. The effects of light on tropisms of urediospore germ tubes and on the initiation of infection were investigated. In addition, the effects of light, temperature, and moisture on development of rust on oats grown in the field and in the greenhouse were studied. A study was made of the field reaction of oat varieties to crown rust. Field varietal tests were made one year at St. Paul, Minnesota, and two at Baton Rouge, Louisiana. In studies on the nature of resistance of oats to crown rust, the effects of pH on growth of urediospore germ tubes, and the effects of extracts from host plants on urediospore germination and germ-tube growth were investigated.

Effects of Temperature on Viability of Urediospores

Fromme (9) obtained a 0.2 per cent germination of urediospores after storage at room temperature for 84 days. Hoerner (15) found that unprotected urediospores lost their viability within 22 days, with a minimum temperature during this period of - 27° F. and a maximum of 42° F.; but spores protected by a 1 foot covering of leaves and snow, under a similar range, remained viable 44 days. Maneval (18) determined that urediospores kept part of the time at room temperature and part of the time in a cool room germinated after 164 days. Melhus and Durrell (21) reported 20 per cent germination after 55 days in urediospores stored at 13° C.; and Sibilia (33) found that age of urediospores determines their germinability; spores 1–3 days old, 80–90 per cent germination; those 15 days old, 50 per cent germination, and urediospores 25 days old germinated 28 per cent.

It seemed necessary first of all to determine the length of time urediospores retain their viability under different conditions. Obviously, if they survive the summer in Louisiana, it would be necessary for them to withstand a considerable period of high temperature, usually coupled with high humidity. Furthermore, it would be necessary for them to survive freezing temperatures at least a few times during the winter.

Rusted oat leaves were placed in manila envelopes and stored in incubators at - 18, - 3, 0, 10, 14, 15, 20, 27, 27.5, 29. 30, and 33 degrees C. Germination tests were made at intervals, in water, at 15°–20° C., the optimum temperature for urediospore germination; and the maximum germination for spores at each storage temperature was recorded. The results are given in table 1.

From these results it is concluded that very few, if any, urediospores of *Puccinia coronata,* exposed continuously to temperatures as high as 27° C., remain viable for more than two and one-half months, and probably not so

TABLE 1.—*The effect of temperature on the viability of urediospores*

Collection	Date collected	No. days stored	Storage temperature and percentage of germination[a]					
			0° C.	10° C.	14°–15° C.	20° C.	27°–27.5° C.	33° C.
Race 17	Oct. 13, 1931	48	45	–	–	–	–	–
Race 1	Jan. 1, 1932	14	65	60	–	65	60	3
		28	37	42	–	35	40	2
		135	22	18	–	0	0	0
Mass field collection	May 5, 1932	50	42	87	40	–	0	0
	May 5, 1933	78	–[b]	15	3	–	tr.[c]	–
		136	–	47	0	–	–	–
		180	–	2	0	–	0	–
		233	–	0	0	–	0	–
		388	–	34	0	–	0	–
		397	–	0	–	–	–	–
		413	–	8	–	–	–	–
		452	–	0	–	–	–	–
	June 19, 1933	19	–	65	48	–	0	–
		60	–	60	0	-	0	–
	Jan. 1, 1934	182	–	9	–	–	–	–
	Feb. 6, 1934	150	–	16	–	–	–	–

[a] At −18°, −3°, +29°, and +30° C., storage temperatures, no spores germinated.
[b] No tests.
[c] Trace.

long as that. On the other hand, indications are that urediospores exposed to temperatures from 0° to 10° C. in nature may retain their viability for a much longer time.

The effects of high temperatures on viability of urediospores of *Puccinia coronata* are extremely important in a consideration of over-summering of crown rust in the southern United States. Spores that will not withstand approximately 27° C. for considerable periods of time could hardly survive the hot southern summers and cause infection the following fall or early winter, unless, perhaps, they were protected inside of straw piles.

That high temperatures are lethal to the spores is very significant. The experiments reported above were made at moderate humidities. Had the humidity been raised, the spores probably would have lost their viability even sooner. If the spores were to survive the southern summers, it would be necessary for them to withstand not only the high temperatures but also the high humidities. The results obtained, therefore, render it doubtful that urediospores can survive the summer in Louisiana.

Epidemiology Studies in Louisiana

It should be borne in mind that crown rust in Louisiana depends entirely on the uredial stage, the other stages being of no importance because of the absence of susceptible alternate hosts. From the experiments on factors affecting the longevity and germination of spores, it is apparent that the really critical factor probably is the ability of the urediospores to survive the long, hot, moist summers. If they survive, then the rust may complete its cycle in that area. If not, it would be necessary that the inoculum be blown in. The observations recorded in this section were directed particularly, therefore, to the questions raised by these considerations.

Field observations on crown rust were made at Baton Rouge for 2 years. The time of the first appearance of rust in winter, the rate of spread, and the environmental conditions prevailing at different times during the two seasons, 1932–1933 and 1933–1934, were studied carefully.

For the season 1932–1933, experimental plots of oats were planted at Baton Rouge on October 12. Conditions for germination were favorable and there was a good stand within 10 days. Oats grew well and the plants were soon several inches high. A careful check was made frequently to determine the first appearance of rust in the plots. No rust was found on November 22, December 2, December 23, or on December 28, although temperature and moisture were favorable to infection during all that time. On December 30 rust was found on volunteer oats plants on the south side of the botany building on the university campus, a distance of about three-quarters of a mile from the oats plots. On January 9, 1933, rust was again found on volunteer oats growing in a protected place on the south side of a seed house in the field, about ½ mile south of the plots. There was a severe freeze on February 8 and 9, the temperature as low as 17° F. Most of the varieties were killed to the ground. Recovery was very slow, and in some varieties only a few plants recovered. On February 28 there was 5 to 10 per cent of rust on susceptible varieties, although some varieties were not yet rusted at this time. From this time on there was a rapid increase in rust infection in the experimental plots. The rust became epidemic during March and the severity increased as the season progressed. The severity of infection on different varieties is indicated in table 6.

An experiment was planned to determine how long urediospores of *Puccinia coronata* would survive in nature at Baton Rouge during the summer. Oats plants severely rusted with uredia and telia were collected on April 11, 1933, and placed under various conditions in an attempt to simulate those in nature. At the beginning of the experiment 65 per cent of the urediospores germinated. The urediospores were exposed 1) on dry sills beneath a raised cotton house in the field, University Farm, Louisiana State University, 2) in a clump of bamboo near the plots, 3) on dry sills in a private garage, and 4) on the ground beneath benches in a greenhouse.

After 75-days exposure, germination tests were made. None of the spores germinated. The urediospores that were scraped from the dry leaves of the plants that had been kept on the dry sills in the two different places were normal in appearance, except for some fading of the orange coloring matter. The plants that had been left on the ground in the greenhouse and out of doors were well disintegrated. Very few normal spores could be scraped from the remains of the leaves, most of them having been wholly or partially destroyed by insects or other organisms.

In urediospore material that had been stored inside for approximately the same length of time, 78 days, at 10, 14, and 27 degrees C., the percentages of germination were 15, 3, and trace, respectively.

From the above results it is concluded that in the vicinity of Baton Rouge, Louisiana, urediospores lose their effectiveness as inoculum in a relatively short time in nature. This loss of viability may be attributable to spores having germinated, (abundant rain and favorable temperature suggest this explanation); to destruction of spores by other organisms; or to loss of viability because of high temperature and high light intensity. The effects of high temperatures on viability of urediospores of *Puccinia coronata* are shown in table 1.

Careful surveys were made at Baton Rouge in the summer of 1933 to determine whether volunteer oats or rust-susceptible grasses survive the high summer temperatures. No volunteer oats was found from July 1 to October 1; in fact, oats planted in the greenhouse in July did not germinate. No cultivated or wild grasses susceptible to crown rust were found during the same 3-month period. Corn (*Zea mays*) was the only gramineous plant rusted on July 17, and corn and Johnson grass (*Sorghum halapense*) were the only grasses observed rusted on September 14, 1933. Neither the rust of corn, nor the rust from Johnson grass attacks oats. It is thus obvious that if there are no green hosts harboring the crown rust organism during the summer in Louisiana, and if urediospores lose germinability when exposed in nature the inoculum for initial infection in the fall or winter must be blown in from other regions.

There is a possibility, however, that urediospores of *Puccinia coronata* might oversummer inside of straw piles, where they would be less likely to germinate or lose their viability because of attack by other organisms. This possibility was investigated in 1933. On September 12, 1933, oats straw was collected from straw stacks near Tallulah, Louisiana, and taken to the laboratory at Baton Rouge. No crown rust was found on straw of the 1933 crop, but teliospores of both *P. coronata* and *P. graminis* were found on the straw of the 1932 crop. Urediospores also were present, but, as none of them germinated, no attempt was made to determine their specific identity.

Oats was again planted at Baton Rouge on October 10, 1933. As in the previous year, the growing season was very favorable and the plants grew well. Observations again were made in order to detect the first appearance of crown rust in the field. Although plants were vigorous and several inches high and conditions were very favorable for infection, no rust was found before about mid-December. During December warm weather prevailed and the plants grew very fast. By December 26 there was nearly 100 per cent prevalence of rust in the plots, and the severity was as high as 10 per cent in many varieties. On January 15, 1934, the rust prevalence was very high and the severity on many susceptible varieties was as high as 60 per cent. Some varieties had headed and on these, especially, telia had developed. On February 6, after two freezes the previous week, no winter injury was apparent. In February the rust became epidemic, increasing in severity as the season progressed. The severity of infection is given in table 6.

The fact that vigorously growing plants of susceptible oat varieties were present in the field at Baton Rouge during two crop years, 1932–1933 and 1933–1934, and did not become rusted before the middle of December, at least two months after the emergence of the plants, is strong circumstantial evidence that crown-rust inoculum was not present at Baton Rouge during October, November, and early December. The source of the first inoculum in the winter is not known, but it is probable that urediospores are blown in to Louisiana from some other section. It is quite possible that the uredial stage, which develops in northern States during the summer and persists there during the fall on vounteer oats and wild grasses, gradually spreads southward with the lowering of temperature, finally reaching Louisiana about mid-December. It is also possible that the rust may oversummer at relatively high elevations in the Southeastern States, or, possibly in Mexico.

Effect of Temperature on Percentage and Rapidity of Urediospore Germination

Cardinal temperatures for germination, as given by different investigators (12, 18, 24, 25, 44) differ somewhat. Figures for the minimum range from 1° to 7° C. For the optimum a range of from 12° to 22° C. has been given. The highest maximum recorded is 35° C.

In the development of epidemics, the effect of temperature on the speed with which spores germinate and on the percentage that germinate is, of course, very important. As moisture often is present for relatively short times only, the shorter the period required for a large percentage of spores to germinate, the better the chances for infection. In the studies on the effect of temperature on the germination of urediospores, therefore, both time and percentage of germination were taken into consideration.

Fresh urediospores were collected from seedlings growing in the greenhouse. Spores were removed from open pustules with a scalpel and brushed on the surface of distilled water in a Syracuse dish. The dishes were stacked one on top of the other and placed immediately in incubators. All glassware used was cleaned thoroughly in potassium-dichromate-sulphuric-acid cleaning solution, rinsed thoroughly in distilled water, and dried with clean cheesecloth. Microscopic examinations were made at intervals and counts were made of germinated spores and percentages of germination computed. The results are given in table 2. These percentages are based on the highest germination count at a given temperature, regardless of the length of time involved. In tabulating composite results of a number of germination trials with the object of striking an average total percentage of germination at a given temperature this was the only method applicable. For example, if total percentages of germination at 0–2, 10, 15, and 20 degrees C., are computed after one hour and ten minutes, it is found that there is 4 per cent germination at 0–2; 55 per cent at 10; 76 per cent at 15; and 89 per cent at

TABLE 2.—*The effect of temperature on the germination of urediospores*

Experiment number	Temperature and percentage of germination						
	−18° C.	0°–2° C.	10° C.	15° C.	20° C.	27.5° C.	33° C.
1	0	58	90	95	95[a]	95[a]	0
2	0	15	80	85[a]	60[a]	55	0
3	0	2	95	86	85	–	0
4	0	18	80	76	89	–	–
5	–[c]	0	78	59	60	3	0
6	–	0	48	65	62	–	–
7	–	66	22	60	40	20	0
8	–	66	30	86	46	26	0
9	–	58	48	76	46	–	0
10	–	80	52	–	6	–	0
11	–	72	40	66	20	22	0
12	–	60	28	82	42	24	0
13	–	54	40	70	48	–	–
14	–	78	42	–	6	–	–
15	–	–	–	65	–	–	tr.[b]
16	–	–	–	55	–	–	tr.
Average	0	27.3	55.4	68.6	55.4	27.4	tr.

[a] Percentage estimated.

[b] tr. = less than 1 per cent.

[c] – = not included in test or no readings made.

20 degrees C. But, after 25 hours, the total germination at 0°–2° is 18 per cent, while at 10° it is about 80 per cent (estimated). The maximum germination at 15° and 20° C. was after one hour and ten minutes. Thus, it is determined that although germination of urediospores at a temperature much lower than the optimum may be abundant if the spores are in water sufficiently long, germination obtains at or near optimum temperature, 15°–20° C., much more promptly. From table 2 it will be seen that the minimum temperature for germination is probably just above the freezing point. The optimum probably lies between 15° and 20° C. The maximum is probably slightly below 33° C.

It is evident from the results that the temperature during the growing season for oats usually would be favorable for the development of crown rust. The optimum lies between 15 and 20 degrees, and spores will germinate at temperatures just above freezing. Consequently, winter temperatures in Louisiana would be quite favorable for the development of rust, and this probably accounts for its rapid development during the winter months.

Effect of Temperature on Rust Development Subsequent to Infection

Having determined the cardinal temperatures for the germination of spores, it next seemed desirable to determine the effect of temperature on the infection of the host plant and on subsequent development of rust. Marland and Kuprianova (19) found that the duration of the incubation period of urediospores of crown rust varies according to the temperature. Thus, with a minimum temperature of 7.2°, mean 10.7°, and maximum 14.3°

C., it lasts 14 days, while at 14.6° minimum, 10.3° mean, and 25.1° maximum it is reduced to 7 days. In experiments under constant temperature conditions, the incubation period of the rust lasted 6 or 7 days at 18° to 20° C. and as long as 9 days at the maximum temperature. Plants of Victory, C. I. 1145, and Gopher, C. I. 2027, oats were grown in the greenhouse, 10 to 15 plants per pot; and, when about a week old, the seedlings were inoculated with fresh urediospores, using the regular procedure of atomizing the plants and transferring the inoculum to both surfaces of the leaf with a scalpel, then atomizing again and transferring to moist chambers for incubation for 48 hours.

TABLE 3.—*The effect of temperature on the development of infection in oat seedlings by urediospores of Puccinia coronata 17*[a]

Experiment No.	Oat variety	No. pots inoc.	Incubation temperature	Post incubation temperature	Rust infection
1, 4, 5	Victory	15	20° C.	10° C.[b]	None in 5 pots; on all plants in 10 pots.
1, 4, 5	Victory	15	20° C.	0°–2° C.	None
1, 2, 3, 4, 5	Victory	10	20° C.	20° C.	Severe on all plants.
2, 3, 5	Victory	15	0°–2 C.	20° C.	None in 8 pots; trace on a few plants in 7 pots.
6	Gopher	6	3° C.	20° C.	8–10 per cent severity on 50 of 75 plants.
6	Gopher	2	20° C.	20° C.	90 per cent severity on all plants.

[a] Incubation period was 48 hours in all experiments.

[b] Plants in 5 pots in experiment 1, post-incubated at 10° C., died after 21 days because there was insufficient light.

From table 3 it will be seen that of the plants incubated at 0°–2° C. for 48 hours and then transferred to the greenhouse at about 20° C. for the remainder of the incubation period (here designated post-incubation period), 10 pots in all, only a few plants in 2 pots developed a few scattered pustules. The checks incubated at 20° C. and kept in the greenhouse at approximately the same temperature during the post-incubation period all rusted rather severely. In the 6 pots of Gopher oats, incubated at 3° C. for 48 hours and transferred to the 20° C. greenhouse, 50 of the 75 plants rusted, the severity being 8–10 per cent, as compared to 90 per cent for the checks. No plants rusted in the 10 pots of Victory oats incubated at 20° C. for 48 hours and then transferred to the 0°–2° C. temperature room for post-incubation. Checks rusted heavily in the greenhouse. In the 10 pots of Victory oats incubated at 20° C. for 48 hours and post-incubated at 10° C., all plants in 5 pots in one experiment rusted, as did the checks at 20° C. in the greenhouse. Plants in the 5 pots in the other experiment died after 21 days because of insufficient light in the temperature room, where there was only one 500-watt light.

Rust developed on some plants incubated at 0°–2° C. and post-incubated at 10° and 20° C., but did not develop on plants incubated at 10° or 20° C. and post-incubated at 0°–2° C. This indicates that the rust invades the host even at low temperatures, but is prevented from developing uredia at low temperatures. Although plants were kept in the 0°–2° C. room upwards of 3 months after inoculation, no rust developed. The fact, too, that urediospores germinate at 0°–2° C. is additional evidence that infection may occur at this low temperature.

The results indicate again that crown rust finds rather congenial conditions for development during the winter in Louisiana. The fact that infection can take place at relatively low temperatures suggests that considerable infection could occur at night when the temperature is moderately low and the rust could then develop rapidly at the higher temperatures during the day. It is obvious, however, that while infection can take place at low temperatures, the development of the rust requires higher temperatures. Daytime temperature, even in winter, is usually sufficiently high to enable the rust to develop rather rapidly. It is concluded, therefore, that temperature is usually favorable for rapid development of the rust in Louisiana.

Effect of Light on Tropisms of Urediospore Germ Tubes

Fromme (10) found that 86 per cent of the urediospore germ tubes of *Puccinia coronata* grew away from the source of light. Germ tubes of controls in darkness grew in all directions. He suggested that these negative heliotropic reactions might be important in causing the germ tubes to enter through stomata.

Dillon Weston (41, 42, 43) found that urediospores of *Puccinia graminis tritici* and *P. graminis avenae* failed to germinate in sunlight or under red, orange, yellow, and purple filters exposed to strong sunlight. Controls in the dark germinated readily. Spores germinated under green and blue filters, and when spores were dusted on the surface of different dye solutions and exposed to sunlight, those on light green germinated but those on Congo red did not. In darkness, spores germinated normally on solutions of the same concentrations. Dillon Weston states that these general conclusions apply also to *P. coronata* and *P. triticina.*

As there is experimental evidence that some rusts cause more abundant infection in light than in darkness, the effect of light on the germination of urediospores and on subsequent infection was studied. While other factors probably are more important in nature than light, there is a possibility that periods of very cloudy weather or of intense sunshine might affect rust development appreciably. First of all, experiments were made on the growth direction response of uredial germ tubes to light of different wave lengths. For comparison several other rusts also were studied.

Spores were allowed to germinate in hanging drops, the procedure being the same as that outlined for studying effects of plant extracts on spore germination. Petri dishes containing the hanging-drop cultures were placed in a partitioned copper box, equipped with Corning light filters in one vertical wall. The apparatus was so placed that white light played on the light filters. In the experiments made in diffuse day-light the box was placed with the filters facing the window. In the experiments made in the 20° C. dark room, a 500-W. light with metal reflector was placed at a distance of approximately 2 feet from the light filters. Checks were set up similarly in hanging drops, and the Petri-dish cultures were set on top of the copper box, exposed directly to white light. Microscopic observations were made at intervals. Table 4 summarizes the phototropic responses of the urediospore germ tubes of *Puccinia coronata avenae, P. graminis avenae, P. graminis tritici,* and *P. triticina,* to white, red, blue, violet, and green light.

From table 4 it will be seen that germ tubes of *Puccinia coronata* respond negatively to white light (See "Check"). There is one exception, that in experiment 6, in which most of the germ tubes were diaphototropic, or at right angles to the incidence of light. The same predominantly negative response is found in the case of blue light. Only occasional germ tubes grew toward the incidence of light. This apparent positive reaction attributed to white light and blue light by a few scattered germ tubes might possibly be a like reaction to moisture rather than light. In some cases there was evidence of germ tubes having turned toward the region of moisture when evaporation had occurred at the margin of the drop. There was a tendency to negative response to violet light in 3 of the 8 experiments on *P. coronata.* The remaining 5 gave no response. There seemed to be no tropic response of urediospore germ tubes of *P. coronata* to green or red light, the growth being in all directions.

With *Puccinia graminis avenae,* there was slight germination in white light; response of the germ tubes was, however, predominantly negative. In blue light, germination was better and the phototropic response was predominantly negative; but some germ tubes grew toward the incidence of light. The response to violet light was decidedly negative in 4 of the 6 experiments. Nearly three-fourths of the germ tubes turned away from the incidence of light and one-fourth grew toward it. In 2 of the 6 experiments, however, there was no apparent phototropic response to violet light. There was no apparent response either to red or green light, the germ tubes growing in all directions.

Puccinia graminis tritici and *P. triticina* were very similar in their reaction to light of different wave-lengths; in both rusts there was a decided negative response to white light. With *P. graminis tritici,* the same reaction occurred in blue light in most cases, that is, the response was negative. With *P. triticina* there was a decided negative response in only 2 of the 5 experiments; in 2 of the remaining 3 there was a tendency toward negative response, and in one there was no response to blue light. The germ tubes of *P. graminis tritici* branched profusely; the branches, however, seemed not to respond to light but grew in all directions, regardless of the kind of light. Germ tubes of *P. graminis tritici* showed no apparent response to red, vio-

TABLE 4.—*Phototropic response of urediospore germ tubes to light of different wave lengths*[a]

Rust species	Exp. No.	Kind of light[b] and response[c] of germ tubes				
		Check: white	Red	Blue	Violet	Green
Puccinia coronata avenae	1	−	No response	−	−; +−; and +	No response
	2	−	do.	−	do.	do.
	3	−	do.	−	46% −; 47% +−; 7% +	24% −; 54% +−; 22% +
	4	−	do.	−; few +	No response	No response
	5	−	do.	−	do.	do.
	6	+−	do.	−	do.	do.
	7	−	do.	−	do.	do.
	8	−	do.	−	do.	do.
P. graminis avenae	1	No response	No response	95% −; 5% +	65% −; 35% +	No response
	2	do.	do.	do.	75% −; 25% +	do.
	3	75% −; 25% +	do.	90% −; 10% +	do.	do.
	4	−	do	do.	do.	do.
	5	−	do.	−	No response	do.
	6	−	do.	−	do.	do.
P. graminis tritici	1	−; some +	No response	−; branches no response	No response	No response
	2	−	do.	do.	do.	do.
	3	75% −; 25% +	do.	90% −; 10% +	do.	do.
	4	−; some +	do.	do.	do.	do.
	5	−	do.	95% −; 5% +	do.	do.
	6	No germination	do.	No germination	do.	do.
	7	−	do.	−	do.	do.
	8	−	do.	No response	do.	do.
P. triticina	1	−; some +	− tendency	− tendency	− tendency	No response
	2	−	do.	do.	do.	do.
	3	−	75% −; 25% +	90% −; 10% +	No response	do.
	4	−	No germination	do.	do.	do.
	5	−	No response	No response	do.	do.

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[a] Temperature was 20° C. in all cases.

[b] Source of white light was a 500-W. lamp at 18 inches.

[c] The tropisms of germ tubes are represented by symbols: −, negative phototropism; +, positive; and +−, diaphototropism.

let, or green light. There was a tendency to negative response. In 3 of 5 experiments on *P. triticina* there was an apparently negative response to red light, no response in one experiment, and only a trace of germination in the remaining one. *P. triticina* showed a tendency to respond negatively to violet light in 2 of the 5 experiments, with no response in the remaining 3. Green light provoked no response.

From the results of these experiments it is concluded that blue light rays, and to a lesser extent the violet, are responsible for the negatively phototropic reaction of germ tubes to white light in the 4 rusts studied.

Effect of Light on the Initiation of Infection

It seems probable that the negative response of germ tubes to light may account partly for the fact that they enter through stomata. However, it is known that stomata are often closed at night, and it seemed desirable, therefore, to find out whether, in the absence of the phototropic effect of light, germ tubes could enter stomata, particularly when they are closed at night. Consequently, experiments to determine this were made.

In preliminary experiments it was determined that almost all the stomata of oats are closed during darkness, even in extremely high atmospheric humidity. If the germ tubes of *Puccinia coronata* are able to penetrate closed stomata, we might expect almost as great severity of infection when plants are in darkness as under normal conditions of alternating light and darkness, with long periods of high atmospheric humidity. If entry is through the occasional stomata that remain open, we might expect the severity of infection in darkness to be very much lower than normal.

The work reported here was a part of more extensive experiments, made with a number of rusts, and reported in a separate paper (14), of which the writer was the junior author.

In the work on crown rust of oats done in the winter of 1931–1932, seedlings of the varieties Gopher (C. I. 2027) and Victory (C. I. 1145) were inoculated with physiologic races 1 and 17 of *Puccinia coronata.* Both varieties are susceptible to both rust races. The "light series" was run in the greenhouse at a temperature range of 18°–25° C., averaging about 21° C. Some of the "dark series" were run in a photographic dark room, where the temperature usually was 20°–22° C. An electric fan circulated the air in the dark room and conditions remained fairly uniform and comparable with those of the "light series" throughout the experiments. Others of the "dark series" experiments were made in a heavy zinc box, so constructed that light was excluded, but with no facilities for circulation of air.

Most of the inoculations were made after dark in the evening, when one could be fairly certain that the stomata were closed. In some cases plants had been in darkness 1 to 3 hours before they were inoculated; more often they had been in darkness 5 to 6 hours before inoculation; and in some cases plants were kept in darkness 8 to 12 hours or longer before inoculation. Plants were inoculated in the usual manner, as described earlier in this paper, then were kept in moist chambers for varying lengths of time, usually 12 to 24 hours, and occasionally as long as 72 hours. For the "dark series" the plants were in darkness throughout the moist-chamber period, and usually the plant surfaces were allowed to dry before the plants were returned to the light. In the "light series," conditions were more or less comparable to field conditions, the inoculated plants being placed in moist chambers during the night and then exposed to alternating periods of darkness and light. At the end of the moist chamber period, plants were returned to the greenhouse bench until notes on infection could be taken. Ten days usually was sufficient time for the eruption and normal development of uredia. The prevalence of infection (percentage of plants infected), and the severity of infection (the abundance, size, and distribution of uredia over a leaf surface) were determined and recorded.

The Prevalence of Rust Infection in Light and Darkness. Results indicate that darkness during incubation did not hinder infection of oats by *Puccinia coronata*, the prevalence of rust on Gopher oats in darkness being approximately the same as in light, and the prevalence on Victory oats being slightly higher in darkness than in light (Table 5). For considerable periods, however, darkness had a decided effect on Victory oats seedlings and the prevalence of crown-rust infection. After 18 to 48 hours in darkness before inoculation and 58 to 60 hours in darkness after inoculation, oats seedlings were severely etiolated and the tips of many of the leaf blades were dying. The prevalence of crown-rust infection on such plants was reduced 56.7 per cent, and most of the infections were in the basal region of the leaf blade, the part that recovered first from severe etiolation. The results of these experiments probably are to be attributed to the effect of darkness on host metabolism, not to its effect on stomatal behavior and entry of the parasite.

TABLE 5.—*The effect of light and darkness on initial infection of oats by Puccinia coronata, as measured by the prevalence and severity of rust on plants of two varieties kept in dark or light for varying periods prior to and subsequent to infection*

Host	Light conditions in moist chamber	Prevalence of infection			Severity of infection	
		No. plants inoculated	No. plants infected	Per cent infected	Per cent plant with light infection	Per cent plants with heavy infection
Gopher oats	Light	75	73	97.3	40.0	57.3
	Dark	165	163	98.7	36.3	62.4
Victory oats	Light	377	335	89.0	12.8	76.2
	Dark (short)	135	125	92.6	33.3	59.3
	Dark (long)	236	134	56.7	56.7	0.0

The Severity of Rust Infection in Light and Darkness. Light or its absence did not affect the severity of crown rust on Gopher oats; but, on Victory, darkness slightly reduced the proportion of severely rusted plants and increased the proportion of lightly rusted plants (Table 5). Darkness in excess resulted in very light rust infection, but, as discussed previously, this was to be attributed to the etiolation and poor vigor of the host.

Nature of Resistance of Cereals to Rust Fungi

A very comprehensive review of the literature relating to the nature of resistance of cereals to *Puccinia graminis* has been given by Hart (12). Her own investigations showed that, in addition to morphologic and protoplasmic resistance, there is a third type of resistance of cereals to rust, namely, functional resistance (11). Cobb (6) was the originator of the theory of morphologic resistance to rust. Ward (39) concluded that there was a protoplasmic antagonism between host and pathogen. The work of others, particularly Stakman (35), supported Ward's conclusion. The work of Stakman (34) and Hursh (16) proved conclusively that morphologic characters sometimes were responsible for resistance. The studies of Marryat (20), Newton (27), Allen (1, 2, 3), Ruttle and Frazer (31), and Wellensiek (40), supported the view of a protoplasmic resistance. Leach (17) advanced the theory that specific nutrients necessary to the development of the stem rust fungus may be present only in susceptible plants and absent in resistant or immune plants. Ezekiel (7) found that extracts from different wheat varieties differed in their ability to support the growth of physiologic forms of *P. graminis tritici* in precise agreement with the respective resistance or susceptibility of the variety to the various physiologic forms of stem rust. Compatability of physiologic forms with the host varieties was indicated almost universally by greater length of urediospore germ tubes in extracts from susceptible varieties, by less branching, and by more infrequent production of apical swellings than in extracts obtained from resistant varieties. Sharvelle (32), working on flax rust, found that extracts obtained from different varieties "supported the growth of urediospore germ tubes of the rust organism in precise agreement with the rust reactions of the varieties from which they were obtained." The same worker obtained indications that morphological characters and stomatal behavior might also contribute to resistance of flax to rust.

From the observed facts regarding the epidemiology of crown rust in Louisiana, it is clear that nothing can be done to reduce the amount of inoculum. Susceptible species of the alternate host are not present; consequently, reduction in amount of inoculum by eradicating the alternate host is not possible. Proper fertilization of the soil might possibly reduce the amount of rust somewhat, but, from previous experience with cereal rusts, it appears that this method would be merely palliative. The one method for reducing rust losses that would seem to have some chance of success would be the development of resistant varieties. The existence of numerous physiologic races of the rust organism has been demonstrated by a number of workers, including Hoerner (14), Popp (30), Parson (28), Frenzel (8), Murphy (22, 24), Peturson (29), Brown (4), and Straib (38).

In the present work, a large number of varieties were tested in the field at St. Paul, Minnesota, for a year and at Baton Rouge, Louisiana, for 2 years; but, because the rust develops so abundantly in Louisiana and the inoculum apparently is blown in from the outside, it seems likely that there would be a good random sample of physiologic races in the field. The situation is quite different from that in isolated regions where the rust persists throughout the year in the uredial stage. In such regions only a few physiologic races might predominate. It has been the experience with other rusts, however, that there are likely to be many races in a region into which the rust is blown from extensive areas. It seems quite probable, therefore, that the tests in Louisiana are particularly significant.

Tests of Varietal Resistance

The resistance of Victoria, Bond, Alber, Berger, and other varieties has been noted by Murphy and Stanton (23), Stanton and Murphy (36), Murphy (22), Murphy, Stanton, and Coffman (26), and others. These workers have obtained promising results in breeding for resistance to *Puccinia coronata, P. graminis avenae, Ustilago avenae,* and *U. levis.*

The field reaction of about 100 varieties of oats to crown rust was determined over a period of 3 years. The first tests were made at St. Paul, in 1932, while those of 1933 and 1934 were made at Baton Rouge. Many more varieties were tested at Baton Rouge than at St. Paul. In Minnesota the varieties were spring-sown, while, in Louisiana, they were sown in October. The amount of crown rust inoculum at St. Paul early in the season of 1932 was limited, and the rust notes were taken late in the season, when the plants were nearing maturity. At Baton Rouge the rust was epidemic both years (1932–1933 and 1933–1934). Abundant inoculum was present in the season of 1932–1933, from late February until the oats were harvested in late May. In 1933–1934, inoculum was abundant from December 26, 1933, until harvest in May, 1934. All varieties grown at Baton Rouge were exposed equally to the inoculum, as the plots, consisting of three rod rows in most cases, were replicated systematically 2 or 3 times.

The reaction of the varieties in the field is given in table 6. The rust estimates given are the final estimates made on each variety. With most of the varieties at least 2 readings were made at different times. The degree of rust infection was estimated according to the scale for estimating rust percentages adopted by the Division of Cereal Crops and Diseases, U. S. Department of Agriculture. As some varieties matured much earlier than others, final readings on all varieties could not be made at the same time. The readings given in table 6, therefore, are not always directly comparable. At St. Paul in 1932 the amount of rust and host reaction only were recorded.

The variety Victoria was very resistant under all conditions, as was indicated by pronounced necrotic flecks and very small pustules. Bond was rust-free in the field at Baton Rouge, both in 1933 and 1934, a fact attributable to the possible absence of physiologic races 33 and 34 to which Bond is susceptible (23, 24, 36).

TABLE 6.—*Severity of crown rust infection on varieties of oats grown in the field at St. Paul, Minnesota, and at Baton Rouge, Louisiana, in 1932, 1933, and 1934*

Varieties tested	C.I. No.	Percentage of rust infection and host reaction							
		St. Paul		Baton Rouge					
		1932		1933			1934		
		Am't.[a]	React.[b]	Preval.[c]	Am't.[a]	React.[b]	Preval.[c]	Am't.[a]	React.[b]
Alber	2766	—	—	—	—	—	—	20	R
Anthony	2143	15	S	100	80	S	—	—	—
Appler (Arkansas)	—	—	—	—	—	—	—	40	VS
Appler (Ga. 200-10)	—	—	—	—	—	—	—	40	VS
Appler (Texas 1401-24)	—	—	—	—	—	—	—	40	VS
Arkansas 19179	—	—	—	—	—	—	—	45	VS
Avoine Guelma 1	—	10	S	100	80	S	—	—	—
Avoine Guelma 2	—	30	S	100	80	S	100	40	S
Avoine Guelma 4	—	25	S	100	70	S	100	40	S
Belar	2760	10	S	100	90	S	100	40	S
Berger	2926	—	—	—	—	—	—	15	R
Bicknell	3218	—	—	—	—	—	—	65	VS
Black Mesdag	1765	80	VS	95	80	S	—	—	—
Bond	2733	—	—	0	0	I	0	0	I
Burke	3042	—	—	—	—	—	—	25	S
Burt	2043	15	S	100	80	S	100	90	S
Canadian	1625	60	S	90	60	S	—	—	—
Carleton	2378	—	S	80	5	S	—	—	—
Columbia	2820	—	—	—	—	—	—	50	VS
"Common of the Country"	2861	—	—	—	—	—	—	40	S
Country Common	2867	—	—	—	—	—	—	40	S
Culberson	273	—	—	—	—	—	—	65	VS
Custis	2041	—	—	—	—	—	—	70	VS
Dun	3041	—	—	—	—	—	—	60	S
Dwarf Culberson	748	—	—	—	—	—	—	35	VS
Early Champion	1623	55	VS	75	25	S	—	—	—
Ferguson 922	2150	—	—	—	—	—	—	50	VS
Fraizer	2381	—	—	—	—	—	—	50	VS
Fulghum	708	30	S	96	60	S	—	60	VS
Fulghum (Ga. 100-58)	—	—	—	—	—	—	—	60	VS
Fulghum (Winter type sel. 699-202)	2498	—	—	—	—	—	—	60	VS
Fulghum (Ferguson Seed Farms)	—	—	—	—	—	—	—	50	VS
Glabrota	2630	20	R	50	10	S	—	—	—
Glen Innis (Minn. Acc. No. 744)	—	90	VS	90	30	S	—	—	—
Glen Innis (Minn. Acc. No. 745)	—	90	VS	100	80	S	—	—	—
Gopher	2027	45	VS	90	30	S	—	—	—

[a] Am't. (amount) = percentage severity.

[b] React. = Degree of susceptibility as shown by host reaction. —, no reading made; S, susceptible; R, resistant; VR, very resistant; VS, very susceptible; SR, semi-resistant; I, immune.

[c] Preval. = Percentage of plants showing infection.

TABLE 6.—(*Continued*)

Varieties tested	C.I. No.	Percentage of rust infection and host reaction							
		St. Paul		Baton Rouge					
		1932		1933			1934		
		Am't.[a]	React.[b]	Preval.[c]	Am't.[a]	React[b]	Preval.[c]	Am't.[a]	React.[b]
Green Mountain	1892	15	S	100	60	S	—	—	—
Green Russian (Minn. Acc. No. 713)	—	30	S	100	5	SR	—	—	—
Green Russian (Minn. Acc. No. 677)	—	40	S	100	90	S	—	—	—
Hajira	1001	35	—	100	90	S	—	—	—
Hastings one hundred bushels	2462	—	—	—	—	—	—	60	VS
Hawkeye	2464	60	S	100	70	S	—	—	—
Iogold	2329	70	S	95	30	S	—	—	—
Iomine	2827	—	S	100	90	S	—	—	—
Iowa D67	2870	70	S	100	85	S	—	90	S
Iowa 444	2331	5	S	100	80	S	—	—	—
Iowa 1224	2871	10	S	100	90	S	—	—	—
Iowa 1257	2872	40	S	80	60	S	—	—	—
Iowar	847	45	S	100	90	S	—	—	—
Joanette	1880	25	—	100	80	S	—	—	—
Kanota	839	80	S	95	80	S	—	50	VS
Kinvarra A8	3035	—	S	—	—	—	—	25	S
Laggan	3044	—	—	—	—	—	—	60	S
Langgewens	3049	—	—	—	—	—	—	30	S
Lampton	3043	—	—	—	—	—	—	25	SR
Lampton (Minn. Acc. No. 743)	—	95	VS	95	25	S	—	—	—
Lee	2042	—	—	—	—	—	—	60	VS
Liberty (Hull-less) (Minn. Acc. No. 676)	—	70	S	80	10	S	—	—	—
Markton	2053	95	VS	90	60	S	—	—	—
Markton × Liberty (Hull-less) 11–28–2	—	60	S	95	40	S	—	—	—
McGehee	—	—	—	—	—	—	—	65	VS
Mirrus	2144	35	S	100	80	S	—	—	—
Minn. Double Cross 11–22–220 (Min. Acc. No. 742)	2874	15	S	100	90	S	—	—	—
Monarch	1682	65	S	70	10	S	—	—	—
Navarro	966	85	VS	95	90	VS	—	—	—
Neb. 21	841	5	S	90	50	S	—	—	—
Neb. 517	2885	15	S	100	90	VS	100	70	VS
Neb. 518	2886	40	S	90	30	S	—	—	—
Neb. 519	2887	5	S	100	75	VS	100	90	VS
Neb. 520	2888	—	S	100	100	VS	—	—	—
Neb. 521	2889	—	S	100	20	S	—	—	—
Rusota	2343	10	S	100	—	S	100	95	VS
Green Russian (N. D. 22001)	2893	—	S	100	70	S	—	—	—
Morota	2344	20	S	100	2	VR	—	—	—

TABLE 6.—(*Continued*)

Varieties tested	C.I. No.	Percentage of rust infection and host reaction							
		St. Paul		Baton Rouge					
		1932		1933			1934		
		Am't.[a]	React.[b]	Preval.[c]	Am't.[a]	React.[b]	Preval.[c]	Am't.[a]	React.[b]
Nortex	2382	—	—	—	—	—	—	35	VS
Nortex (Tex. 9235)	—	—	—	—	—	—	—	40	VS
Norton	2501	—	—	—	—	—	—	35	VS
Rainbow	2345	25	S	100	0, 1, 2	R	—	—	—
Red Algerian	2931	—	—	—	—	—	—	35	VS
Red Rustproof	775	—	—	—	—	—	—	40	VS
Red Rustproof (Sel. 518–3)	—	—	—	—	—	—	—	45	VS
Red Rustproof	1079	—	—	—	—	—	—	65	VS
Appler	1815	5	S	100	80	VS	100	70	VS
Red Rustproof (Louisiana)	—	—	—	—	—	—	—	40	VS
Red Rustproof (Tex. 1118–69)	2503	—	—	—	—	—	—	60	VS
Richland	787	20	S	90	30	S	—	—	—
River Plate	3046	—	—	—	—	—	—	40	VS
Rouge d'Algérie	—	50	SR	100	80	VS	100	50	S
Ruakura	2025	10	—	100	90	VS	—	—	—
"Rustless Sel."	724	30	S	100	80	VS	—	—	—
Sidonian	3047	—	—	—	—	—	—	40	S
Silvermine	659	50	S	95	95	VS	—	—	—
Silvermine	1729	65	S	95	95	VS	—	—	—
Smyrna	3048	—	—	—	—	—	—	35	VS
S. Dak. 165 (Hull-less)	2883	60	VS	50	8	S	—	—	—
S. Dak. 334	2884	30	S	90	50	S	—	—	—
Sterisel	2891	25	—	100	70	VS	100	80	VS
Sunrise	982	15	SR	100	70	VS	100	50	S
Swedish Select	134	30	S	100	80	VS	—	—	—
Uruguay	2916	—	—	—	—	—	—	10	R
Victor	803	30	S	95	95	VS	—	—	—
Victoria	2401	5	VR	100	2	VR	100	2	VR
Victory	1145	15	S	100	80	VS	—	—	—
White Russian	2144	10	S	100	90	VS	—	—	—
Avena 64q	2927	—	—	—	—	—	100	2	VR
Avena 64r	2828	—	—	—	—	—	100	2	VR
Avena 64s	2929	—	—	—	—	—	—	30	S
Avena 64t	2930	—	—	—	—	—	—	5	S

Most of the varieties tested at St. Paul in 1932 were susceptible to crown rust. None were immune, and Victoria alone was very resistant. Glabrota, however, was classed as resistant, and Rouge d'Algérie and Sunrise were semi-resistant. There was a very wide range, 5–95 per cent, in amount of infection on different varieties, because inoculum was scarce until late in the season and some early-maturing varieties may have escaped infection.

In Louisiana most varieties were susceptible in 1933; prevalence and severity of infection were high throughout. The severity on some varieties differed in 1933 and 1934. For example, Fulghum, Kanota, and N. D. 20014, were S (susceptible) in 1933 and VS (very susceptible) in 1934; while the reverse is true of Rouge d'Algérie and Sunrise. These two varieties were VS in 1933 and S in 1934. Whether the differences were real or only apparent is impossible to say, as rust estimates cannot be made with mathematical accuracy.

Some varieties were resistant at St. Paul in 1932 and susceptible at Baton Rouge in 1933 and 1934. Also, some varieties, susceptible at St. Paul, were resistant or semi-resistant at Baton Rouge. These differences in reaction of varieties as to time and place probably were due principally to differences in the prevalence of physiologic races, although meteorologic and soil conditions may also have had some influence.

Effect of pH on Urediospore Germination

Stock (37) reported that urediospores of *Puccinia coronata* and *P. triticina* grew best in acid medium (pH 4.6–6.0). Urediospores of *P. graminis,* on the other hand, grew equally well on acid and alkaline media (pH 4.6–7.2). He determined the pH range for several rusts as follows: *P. graminis,* pH 3.5–7.3; *P. dispersa,* 4.3–7.6; and *P. coronata,* pH 4.5–6.5.

It appeared from the field observations that the reactions of varieties to crown rust probably resulted from physiologic resistance, a view supported particularly by the conspicuous necrotic flecks on Victoria. It seemed desirable, therefore, to investigate the effect of pH concentration on the germination of urediospores, particularly since it has been suggested a number of times that the pH concentration of plant juices might account for resistance.

Solutions having pH concentrations of from 4.4 to 7.6, inclusive, were made up of mixtures of $KH_2PO_4 - NaOH$, according to formulae given by Clark (5). For pH values below 4.4, 5/N HCl was added to distilled water. The actual pH of solutions ranging as high as 7.6 was determined by means of the potentiometer and quinhydrone electrode. The pH values above 7.6 were obtained by mixtures of M/5 H_3BO_3, M/5 KCl with M/5 NaOH. The proportions of each of these solutions necessary for making up a solution of a desired pH value were obtained from Clark (5), and the actual values were not checked by the electrometric method. The procedure for making the germination tests was the same as that outlined for testing the effects of temperature on urediospore germination. Germination tests in aqueous solutions of different pH concentrations were made at or near the optimum temperature for germination. At intervals percentages of germination were computed. In table 7 are recorded the percentages of germination of urediospores of *Puccinia coronata* at different pH concentrations of the aqueous solution used. These percentage figures are based on the highest germination count at a given pH value, irrespective of the time required for maximum germination.

Urediospores of *Puccinia coronata* germinated rather freely throughout a pH range of from 4.4 to 7.6, inclusive, except at pH 7.2–7.3, where germination averaged only 2.9 per cent for a total of 3,000 spores counted in 10 tests (Table 7). There was scant germination at the lowest pH concentra-

TABLE 7.—*Effects of pH concentration on the germination of urediospores of Puccinia coronata*

Test number	pH and percentage of germination[a]													
	2.7	3–3.1	3.3	4.4	5.0	6.1	6.7	7.2–7.3	7.6	8.0	8.4	8.8	9.2	Check Distilled water
1				48	51	66	23	7	46					40
2				76	90	80	67	0	42					92
3				20	18	35	8	3	17					37
4				6	65	92	94	3	47					70
5				12	12	17	7	1	20					30
6	0	0	0	11	20	32	60	2	45					36
7		0		1	7	3	5	1	1					18
8			2	17	22	38	53	1	60					85
9	1	3	4	55	35	60	90	3	60	4	3	1	1	90
10	1	3	2	30	70	45	90	8	6	10	tr.	3	1	65
Average percentage germination	0.66	1.5	2.0	27.6	39.0	46.8	49.7	2.9	25.7	7.0	1.5	2.0	1.0	56.3

[a] Based on counts of 300 spores in each test for each concentration.
Blanks indicate no test made.
Tr. indicates less than 1 per cent.

tion, 2.7, at which only .66 per cent of the 900 spores counted in 3 trials germinated. It is thus concluded that this is probably the lowest pH at which spores will germinate. Likewise, 1 per cent germination occurred at the highest concentration, pH 9.2, and slightly more, 2 per cent, at pH 8.8. The optimum pH for germination in these tests was 6.7. As the pH of distilled water was not determined in all cases, the percentage of germination in distilled water, though higher than that in any solution of known pH concentration, was not used in the comparisons.

As spores germinated fairly well under a wide range of pH concentration, it seems unlikely that the pH of juices of different varieties would differ sufficiently to affect resistance appreciably. The rather crude method of determining the effect of press sap from resistant and susceptible varieties on the germination of urediospores and subsequent development of germ tubes was, therefore, tried.

Effects of Extracts from Host Plants on Urediospore Germination and Germ-tube Growth

Seedlings of oats, grown in the greenhouse, were cut off just above the ground, a definite amount of each variety weighed and then ground in a sterilized meat grinder that had been thoroughly washed in hot running water and then in distilled water. The ground material was then steeped in 2.5 times its own weight of sterile, distilled water and placed in the ice box at about 10° C. for 2 hours. The plant juice from these samples was extracted by squeezing the material through several thicknesses of cheesecloth, and then by means of pressure in a hydraulic press equipped with press cups of noncorrosive monel metal. The expressed juice was then filtered through Berkefeld filters that had been sterilized in the autoclave for 2 hours at 15 pounds' pressure. The filtrate was stored in sterile flasks at about 10° C. until used. The concentration of an extract prepared by the above procedure is equivalent to a 28 per cent solution, which could then be diluted with sterile distilled water to a concentration of 10 per cent. Hanging drops were made upon sterile cover glasses supported on glass rings contained in a Petri dish lined with moistened filter paper. All glassware used in these experiments was thoroughly cleaned, washed in alcohol, rinsed in distilled water, and sterilized in the hot-air oven at 150° C. Hanging drops were made in quintuplicate for each extract, and freshly gathered urediospores were brushed onto the surface of the drop by means of a scalpel. Germination counts for 300 spores in each hanging drop were made after 2.5 hours and 22 hours. Checks were run in distilled water. Thus, for each trial a total of 1500 spores were counted for each extract. The results are given in table 8.

While the differences in percentages of germination in extracts from the resistant and susceptible varieties and in the checks after 22 hours are not so marked as at 3.5 hours, the fact remains that the rate of germination is much slower in extracts from resistant varieties than in those from suscep-

TABLE 8.—*Effects of extracts from oats varieties on urediospore germination in Puccinia coronata avenae*[a]

Source of extract	Extract and percentage of germination			
	After 3.5 hours		After 22 hours	
	10 per cent	28 per cent	10 per cent	28 per cent
Check[b]	48.0	46.85	—	—
Bond	31.8	12.66	35.3	15.30
Victoria	38.0	8.80	50.0	13.77
Victory	46.2	20.17	47.3	21.82

[a] A summary of 8 experiments, in each of which 1500 spores in each extract were counted.

[b] Checks in distilled water.

— No counts possible due to excessive germ-tube lengths.

tible varieties and in distilled water (checks). The difference in rate of growth of germ tubes also is striking. In distilled water no counts could be made after 22 hours because the germ tubes were too long, and the germination percentages had to be estimated. Fairly accurate germination counts, however, could be made in extracts, particularly from Bond and Victoria, after 22 hours, the germ tubes being much shorter than in water.

Victoria is highly resistant in both the seedling and more mature stages. Very large flecks are produced in abundance on the leaves, and minute uredial pustules are produced in these large necrotic areas. Bond apparently is immune from crown rust in the field, at least at Baton Rouge.

From the marked inhibitory effect of extracts from Victoria and Bond on urediospore germination, when compared with germination of checks in distilled water, and with germination in extracts from the completely susceptible variety Victory, it is very apparent that there is some substance in the resistant varieties that is unfavorable to urediospore germination. The abnormal shortness and distortion of germ tubes in extracts from resistant varieties also indicates the presence of an inhibitive substance in varieties highly resistant to *Puccinia coronata.* The development of flecks on Victoria and the immunity of Bond in the field at Baton Rouge are further evidence of physiological resistance to crown rust.

DISCUSSION

Results reported in this paper lead to the conclusion that summer survival of urediospores of *Puccinia coronata* in Louisiana is improbable, a deduction based on controlled experiments and field observations. This being true, it is necessary, of course, that inoculum be blown in from other regions. This increases the likelihood that a considerable number of physiological races may be introduced each year.

Other studies reported here contribute to the understanding of factors affecting the rather rapid development of rust in Louisiana. It has been shown that the urediospores germinate under a rather wide range of temperature, that the optimum is relatively low—a temperature range which occurs frequently in Louisiana. It has been shown further that infection can occur at even very low temperatures, but higher temperatures are required for the rapid development of the rust. This makes it possible, of course, for the rust to develop throughout the entire winter, as infection can take place at any time, and there is enough warm weather to permit rapid development. Light probably so affects the stomata as to permit a more ready entrance of the germ tubes and thus bring about rapid increase of infection. It has been shown that the likelihood of severe infection is greater when the light intensity is high than when it is reduced. This can be correlated, of course, with weather conditions in Louisiana during the winter.

Studies thus far made in Louisiana indicate that only the uredial stage need be considered. Nothing can be done, therefore, to control the rust by the eradication of the alternate host, since it does not occur in that region and probably would not become infected were it present. As, with other rusts, it has been shown that soil fertilization is only a palliative, and, since time of planting would have relatively little inhibitive effect on the development of rust in winter oats, the only feasible method of control is adoption of resistant varieties. This has been the experience of planters in Louisiana for a number of years.

The next logical step in our investigation was to inquire into the nature of resistance. As the field evidence indicates that the resistance is of the general physiological type, the first of the two most promising lines of inquiry would be to determine the resistance of the germ tubes to pH concentrations with a view to attempting a correlation of pH of sap of different varieties with resistance. Because of the fact, however, that the rust can tolerate a rather wide range of pH, it seems very unlikely that acidity of cell sap could account for resistance. The next logical step then seemed to be to find out whether the expressed sap would affect germ-tube development. Positive results were obtained, thus corroborating the field evidence that the nature of resistance is physiologic. Consequently, a study of morphologic characters of resistant varieties seems unnecessary. Inasmuch as it was shown also that the germ tubes probably enter through closed stomata, even functional resistance can hardly be operative. This is added evidence for the conclusion that physiologic resistance is probably the only type of resistance operative against *Puccinia coronata,* and that use of resistant varieties offers the only control of this rust in Louisiana.

SUMMARY

Urediospores of *Puccinia coronata* kept at very low temperature, – 18° C., or at very high temperature, 33° C. rapidly lost their germinability. Spores stored at 10° C. germinated after 413 days, 8 per cent germination having been obtained. At 4°, 15°, and 20° C. urediospores lost their viability rather rapidly.

Urediospores exposed to summer field conditions at Baton Rouge, Louisiana, did not germinate after 75 days. No volunteer oats and no wild grasses susceptible to *Puccinia coronata* were found in the vicinity of Baton

Rouge during the 3 months' period of July 1 to October 1, 1933. Oats were planted at Baton Rouge October 12, 1932, and October 10, 1933, but the first rust found in the season of 1932–1933 was on February 6, 1933, and the first found in 1933–1934 was late in December, 1933. The circumstantial evidence is that crown rust inoculum was not present at Baton Rouge during October, November, and early December in 1932 and 1933.

The minimum temperature for germination of urediospores of crown rust is just above the freezing point, as spores germinated at 0°–2° C. The optimum lies between 15° and 20° C. The maximum is probably slightly below 35° C., as only a trace of germination occurred at 33° C.

Rust developed on plants incubated at 0°–2° C. and post-incubated at 20° C., but did not develop on plants incubated at 10° or 20° C. and post-incubated at 0°–2° C. The indication is that the rust gains entrance to the host at very low temperatures but is prevented from developing uredia.

Urediospore germ tubes of the 4 rusts studied, *Puccinia coronata, P. graminis avenae, P. graminis tritici,* and *P. triticina,* responded negatively to white light and blue light. In violet light urediospore germ tubes of *P. coronata, P. graminis avenae,* and *P. triticina* had a tendency in some cases to negative phototropism, while *P. graminis tritici* did not respond to violet light. There was no phototropic response to green light in any of these rusts; *P. triticina* alone responded to red light, the tendency being a negative one. The conclusion is that the blue rays, and to a lesser extent the violet, are responsible for the negatively phototropic response of the sporelings to white light.

Urediospore germ tubes of *Puccinia coronata* seem to enter the host plant with no difficulty, whether the plants are in light or darkness. Darkness during the incubation period favored severe infection of crown rust on Gopher oats, but only slightly affected severity on Victory oats.

Urediospores of the crown-rust fungus germinated rather freely throughout a pH range of 4.4 to 7.6, inclusive; at pH 4.4 there was 27.6 per cent germination and at 7.6, 25.7 per cent germination. The minimum pH at which germination will occur is probably about 2.7; the optimum, 6.7; and the maximum, 9.2.

The resistance of oat varieties to crown rust was studied under field conditions at St. Paul, Minnesota, and at Baton Rouge, Louisiana. The most highly resistant variety in the field during the 3 years' tests was Victoria, which fell in the class of "very resistant." The variety Bond was immune in the field at Baton Rouge in 1933 and 1934.

Extracts from the highly resistant variety Victoria and from the immune variety Bond (immune from attack by the rust forms used) markedly reduced the percentage of germination of urediospores over that in the checks and in extracts from the susceptible variety Victory, and also caused much distortion of germ tubes and delayed the rate of growth of germ tubes.

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MOVEMENT OF TOBACCO-MOSAIC VIRUS IN TOMATO PLANTS

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INTRODUCTION

Results of studies on the spread of tobacco-mosaic virus from primary lesions in tomato plants were published by Samuel (5) in 1934. The facts presented favored the theory of a slow cell-to-cell movement through plasmodesmata combined with rapid movement through the phloem. They also favored the view that virus travels with the food stream and that determinations on rate and direction of movement of virus might serve to indicate rate and direction of movement of metabolites. Two conclusions of special interest were stated: (1) "When virus passes out from an inoculated leaf, it travels first to the roots of the plant with such speed that it can seldom be intercepted at intervening positions" and "usually about a day later it travels with equal rapidity to the top of the plant." (2) In the early stages virus is transported as separated particles that in some instances are considerable distances apart. The first conclusion seemed important because it implied that neither virus nor metabolites pass from a leaf to the tip of a tomato plant, except *via* the roots. The second seemed important because it was incompatible with the view that tobacco-mosaic virus, during early stages of invasion, moves through tomato stem tissues by multiplying itself along, as has sometimes been assumed. If the conclusion is correct, this movement cannot result from propagation by means of an autocatalytic reaction. Initial spread through stems must be accomplished by movement of virus particles.

Shortly after Samuel's paper appeared, a few experiments designed to confirm his conclusions were undertaken. The results obtained were in general those to be anticipated from Samuel's work, but they did not confirm the view that movement of virus from an inoculated leaf to the tip of a plant is *via* the roots. In the hope of obtaining conclusive evidence on this point and further evidence on rate and direction of movement of virus, and on the ability of virus particles to pass through pieces of stem tissue without infecting them, a number of experiments were made. The purpose of the present paper is to report and discuss some of this work.

MATERIALS AND METHODS

The tomato variety Bonny Best and ordinary tobacco-mosaic virus were used in most tests. Plants were grown in 6-inch pots filled with rich earth. Experiments were conducted in a greenhouse held at about 75° F. Flower buds were removed in order to prevent development of fruits on the plants. Except for slight modifications, the methods adopted for inoculating and testing plants and for recording data were those used by Samuel. Instead of immediately testing the several parts into which plants were cut, Samuel incubated the pieces in test tubes for 1 week before inoculating their juices to leaves of *Nicotiana glutinosa* L. In some cases he set the pieces in soil and allowed them to produce plants that were later observed for symptoms of tobacco mosaic. The chief advantage of this method of testing is that the period of delay permits virus, which might be present in some sections in amounts too small to be detected at time of cutting, to multiply and thus become readily detectable.

In my experiments, inoculations on tomato plants were made by rubbing fresh juice from a mosaic-diseased tobacco plant over the upper surface of the tip leaflet of a leaf about halfway up or somewhat more than halfway up the plant. In certain experiments all plants were inoculated at about the same time, starting at 9:30 a.m. or 12 noon, and were cut up after varying intervals. In others they were inoculated at different times but were cut up at about the same time, beginning at 8 a.m. or 12 noon. Usually not more than 20 minutes were required for either operation. Sketches showing the height of plants and the number and position of nodes were made.

Stems were cut into nodal and internodal sections. A piece about 1 cm. long was taken from each internode midway between the nodes. Two pieces of about the same length were cut from the petiole of the inoculated leaf of each plant. One was taken a short distance below the terminal leaflet; the other near the base of the leaf. In some of the experiments only the internodal sections were kept for testing; in others all sections were retained. Internodal sections were incubated in test tubes, while nodal sections, when not discarded, were set out in a mixture of sand and peat in flats. The internodal sections were tested for presence of virus after 7 to 10 days' incubation by subinoculating to leaves of *Nicotiana glutinosa*. In case nodal sections had been retained, they were sorted into two lots as soon as infection records for internodal sections became available. Cuttings that seemed of special interest because they had adjoined internodal sections through which virus had passed without causing infection, and a limited number of cuttings that had not adjoined such sections were retained. All others were discarded. This was done in order to limit the number of plants to be grown. When rooted, the sections retained were set out in soil in 6-inch pots along with an equal number of cuttings from check plants. As soon as a plant from a cutting showed symptoms of tobacco mosaic, it was discarded. Plants that remained healthy were held under observation for at least 3 months. They were then tested for presence of virus by subinoculating from their tips in the usual way. This was done in order to determine with certainty whether or not the plants were virus-free at the end of the 3-month period. Uninoculated check plants equal in number to the inoculated plants were included in each experiment. They were cut and tested in the same manner as were inoculated plants. Since none became infected, records for check plants have not been presented in the tables.

It was found in preliminary experiments that the interval between time of inoculation and the time when virus moved out of inoculated leaflets varied greatly with different individuals. Although care was taken to select vigorous plants of about the same size for each experiment, and all leaflets selected for inoculation were thoroughly and uniformly rubbed with inoculum, irregularity in time of movement persisted. It was finally decided that the variability might be due to hereditary differences in the plants and that it might be remedied by using individuals belonging to a clone. Accordingly, plants propagated by means of cuttings from a single seedling were grown. All tomato plants used in the experiments to be reported were of this clone, but it cannot be said that they gave more uniform results than the seedlings used previously.

EXPERIMENTS

Cutting and Testing for Virus

Experiments with seedlings had shown that, under the conditions obtaining, virus sometimes moved out of inoculated leaflets in as short a period as 44 hours. It was thought that movement might occasionally begin in less than 44 hours, and several experiments, which will not be reported in detail, were made to test this possibility. In none of 30 plants tested was there movement in 42 hours. No tests were made of plants held for periods longer than 42 hours but shorter than 44 hours. The experiments reported were made after the approximate time required for movement of virus out of inoculated leaflets had been determined.

The data in table 1 are from experiments 1–15, inclusive. Internodes were lettered from the tip downward. The heavy lines in this and other tables show the points of attachment of inoculated leaves. Petiole sections indicated by the letter x were nearest the stems. Those indicated by the letter y were nearest inoculated leaflets. Infected sections are indicated by plus signs; uninfected sections by minus signs. Records for all inoculated plants used in the first 3 experiments and for 35 of 158 inoculated plants used in 12 other experiments are presented. In the plants for which no records are given, virus was either not found or was present in all parts tested. Records for 8 plants (Nos. 15, 21, 23, 24, 28, 30, 31, and 33 of experiments 4–15), in which virus reached all sections, are included.

The data indicate that movement did not occur in 7 (Nos. 1 and 4 of experiment 1, Nos. 1, 3, and 4 of experiment 2, and Nos. 2 and 3 of experiment 3) of the 30 plants used in experiments 1, 2, and 3. In approximately one-half of the 23 plants in which there was movement (Nos. 2, 5, 6, and 10 of experiment 1, Nos. 5, 6, 9, and 10 of experiment 2, and Nos. 1, 5, 6, and 8 of experiment 3) virus had traveled both up and down the stem. In most of these plants it had gone farther down than up, but in Nos. 2 and 5 of experiment 1 and in No. 6 of experiment 3 it had gone farther up than down. In only one case (No. 7 of experiment 1) was virus found above but not below the point of attachment of the inoculated leaf. In 9 of the 23 plants (Nos. 3 and 8 of experiment 1, Nos. 2, 7, and 8 of experiment 2, and Nos. 4, 7, 9, and 10 of experiment 3) movement had been downward only. Virus reached the tip internodes of only 3 plants (No. 2 of experiment 1 and Nos. 1 and 8 of experiment 3).

TABLE 1.—*Distribution of virus in the internodes of stems and petioles of inoculated leaves of tomato plants after different intervals following inoculation*

Experiment	Plant No.	Interval in hrs.	Internode																				Petiole section		Height of plant in inches
			a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	x	y	
1	1	44	-	-	-	-	-	-	-	-	-	-											-	-	13
	2	"	+	-	+	-	-	+	+	-	-	-											-	-	13
	3	46	-	-	-	-	-	-	-	-	-	+											-	+	14
	4	"	-	-	-	-	-	-	-	-	-	-											-	-	14
	5	48	-	-	+	+	+	-	-	-	-	-	-										-	+	14
	6	"	-	-	-	+	+	+	+	+	+	+											-	+	14
	7	50	-	+	-	-	-	-	-	-	-	-											-	+	15
	8	"	-	-	-	-	+	+	+	+	+	+	-										-	-	14
	9	52	-	-	-	-	-	-	-	-	-	-											+	-	13
	10	"	-	-	+	+	+	+	+	+	+	+	+										+	+	15
2	1	44	-	-	-	-	-	-	-	-	-	-											-	-	13
	2	"	-	-	-	-	-	-	-	+	-	-											-	-	12
	3	46	-	-	-	-	-	-	-	-	-	-											-	-	13
	4	"	-	-	-	-	-	-	-	-	-	-											-	-	12
	5	48	-	-	-	+	+	+	-	-	+	+											+	-	12
	6	"	-	-	-	+	+	+	-	-	-	+	-										+	+	13
	7	50	-	-	-	-	+	+	+	+	+	+	+	+	+								+	+	13
	8	"	-	-	-	-	+	+	-	+	-	-	-										+	+	12
	9	52	-	-	+	+	+	+	+	+	+	+	+	+									+	+	11
	10	"	-	-	+	+	+	+	+	+	+	+	+										+	+	13
3	1	48	+	+	+	+	-	+	+	+	+	+	+	+									+	+	13
	2	"	-	-	-	-	-	-	-	-	-	-	-	-									-	-	13
	3	50	-	-	-	-	-	-	-	-	-	-	-	-									-	-	13
	4	"	-	-	-	-	-	-	+	-	-	-	-	-									+	-	14
	5	52	-	-	+	+	+	+	+	+	+	+	+	+									+	+	13
	6	"	-	+	-	-	-	+	-	+	-	-	-										+	+	13
	7	54	-	-	-	-	-	-	-	+	-	-	-										+	+	14
	8	"	+	-	-	-	-	-	-	-	-	+											-	+	13
	9	56	-	-	-	-	+	+	+	+	+	+											+	+	14
	10	"	-	-	-	-	-	+	+	+	+	+	+										+	+	12

TABLE 1—(*Continued*)

Experiment	Plant No.	Interval in hrs.	Internode																				Petiole section		Height of plant in inches
			a	b	c	d	e	f	g	h	i	j	k	l	m	n	o	p	q	r	s	t	x	y	
4 to 15	1	48	-	-	-	-	+	+	-	-	-	-	-	-	-	+	-	-					+	+	29
	2	58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-				-	+	41
	3	62	-	-	-	+	-	+	+	+	-	+	-	-	-	-	-	-	-				+	+	42
	4	"	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-				-	-	44
	5	"	-	-	-	-	-	-	-	-	-	-	-	-	-	-							-	+	29
	6	63	-	-	+	-	-	-	-	-	-	+	-	-	-								+	+	28
	7	64	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-						+	+	27
	8	65	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+					+	-	28
	9	66	+	+	+	+	+	-	-	+	+	-	+	+	-	-							+	+	28
	10	"	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-				+	+	40
	11	"	-	-	-	-	+	+	+	-	+	+	-	-	-	+	-	-	-	-	+		+	+	40
	12	67	-	-	-	-	-	+	-	-	-	-	-	-									+	-	28
	13	70	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-						-	-	30
	14	71	-	-	-	+	+	+	+	-	-	+	+	+	+	+							+	+	28
	15	72	+	+	+	+	+	+	+	+	+	+	+	+	+								+	+	28
	16	73	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+						+	+	28
	17	74	-	+	+	+	+	+	+	+	+	+	+	+									+	+	27
	18	"	-	+	-	-	-	+	+	+	-	-	-	-	-	-	-	-					-	-	30
	19	76	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-					-	-	29
	20	"	+	+	+	+	+	+	+	+	+	+	+	+	+	-							+	+	27
	21	78	+	+	+	+	+	+	+	+	+	+	+	+	+								+	+	26
	22	"	-	-	-	-	-	-	-	-	-	-	-	-	-	-							-	+	29
	23	80	+	+	+	+	+	+	+	+	+	+	+	+	+								+	+	27
	24	"	+	+	+	+	+	+	+	+	+	+	+										+	+	31
	25	82	-	+	+	+	+	+	+	+	+	+	+										+	+	27
	26	"	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+					+	+	30
	27	87	-	+	+	+	-	+	+	+	+	+	+	+	+	+							+	-	26
	28	89	+	+	+	+	+	+	+	+	+	+	+	+	+	+							+	+	26
	29	90	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-				+	+	31
	30	91	+	+	+	+	+	+	+	+	+	+	+	+	+	+							+	+	25
	31	93	+	+	+	+	+	+	+	+	+	+	+	+	+	+							+	+	25
	32	94	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-				+	+	32
	33	95	+	+	+	+	+	+	+	+	+	+	+	+	+								+	+	26
	34	96	-	+	+	+	+	+	+	+	+	+	+	+	+	+							+	+	32
	35	110	-	+	+	+	+	+	+	+	+	+	+	+	+	+							+	+	27

The other 12 experiments showed similar types of movement after longer periods of time in plants that were older and larger than those used in the first 3 experiments. In plants Nos. 2, 5, and 22, virus apparently had just started to move, for it was found only in sections of petiole nearest the inoculated leaflets. One of the plants (No. 15), in which virus was found in every section, was cut only 72 hours after inoculation, while one (No. 32), in which it had just started to move, was cut 94 hours after inoculation. Virus had moved both up and down in the stems of 17 plants. In 2 of the 17 it had moved farther up than down, while in the remainder it had moved farther down than up. In 8 plants (Nos. 1, 4, 7, 8, 11, 12, 13, and 19) it had moved downward only. It reached the tip internodes of 11 plants and the basal internodes of 17 plants.

These experiments provided abundant evidence that virus can move through tissues without infecting them. Records from plants Nos. 3 and 7 of experiment 1, 2 of experiment 2, 8 of experiment 3, and 6, 8, 11, 13, and 18 of experiments 4–15 are particularly interesting in this connection. In all of these, virus passed through two or more serially located internodal sections without infecting them. In plant No. 3 of experiment 1 it passed downward through 5 successive internodal sections without infecting. In plant No. 8 of experiment 3 it passed upward through 3 sections and downward through 5 sections without infection. In plants Nos. 2 and 8 of experiment 1, 2 of experiment 2, and 4, 13, 18, and 19 of experiments 4–15, it passed both sections of petiole without infecting.

Since the roots of the plants used in experiments 1–15 were not tested for presence of virus, no direct evidence was obtained either for or against the hypothesis that movement from an inoculated leaf to the tip of a plant is *via* the roots. However, the speed with which virus traveled to the tips of certain plants brought indirect evidence against the hypothesis. Experiment 16 was designed with the object of securing direct evidence on this question. At the time sections were cut, stumps and roots were left undisturbed. When, after the usual incubation period, the internodal sections were tested, the roots were washed, ground in a mortar, and likewise tested. Table 2 shows the results obtained.

The plants were inoculated between 12 o'clock noon and 12:20 p.m. They were harvested after the periods shown in the table. Ten plants were cut at the end of each period except the first. One plant in the first group was injured in handling and was discarded. It will be seen that virus had moved in 4 of the 9 plants of the first group and in 5, 2, 8, 8, 7, 7, and 8 plants of each lot of 10 cut after 46, 48, 50, 52, 54, 56, and 58 hours, respectively. Virus failed to move in approximately one-third of the plants.

In 5 of the 49 plants (Nos. 2, 9, 18, 42, and 57) in which virus moved, it had reached all parts tested. Two of these plants were from the group cut 44 hours after inoculation. In 34 (Nos. 13, 19, 20, 28, 30, 31, 32, 33, 34, 36, 37, 39, 40, 45, 47, 48, 51, 52, 56, 59, 62, 63, 64, 65, 67, 69, 70, 71, 72, 73, 74, 77, 78, and 79) of the remaining 44 plants, virus had moved both upward and downward in the stems, but in 10 of these (Nos. 19, 30, 31, 34, 45, 47, 62, 64, 65, and 69) it was not found in the roots. In 3 of the 10 plants (Nos. 34, 64, and 69) it was present in tip internodes. In 2 plants (Nos. 14 and 16) it had moved upward only, and in 7 plants (Nos. 3, 5, 43, 44, 49, 54, and 55) downward only. It failed to reach the roots of 2 (Nos. 45 and 55) of the 7 plants in which it had moved downward only. In 1 plant (No. 68) it was recovered from a petiole section only. Evidence against the hypothesis of upward movement *via* the roots was furnished by the 12 plants in which virus was found above the points of attachment of inoculated leaves but not in the roots. It failed to infect one or more sections through which it passed in 32 of the 49 plants allowing movement. Two of the plants (Nos. 5 and 49) are of special interest because virus passed into the roots of each without infecting any of the intervening sections tested.

The record of experiment 17 presented in table 3 brings data from nodal as well as internodal sections and roots. The table shows that virus had moved out of the inoculated leaflets of 22 of the 32 plants. It reached the stems of all plants in which it moved except No. 6. Movement occurred in 1, 2, 2, 3, 3, 4, 4, and 3 of the 4 plants cut after 46, 48, 50, 52, 54, 56, 58, and

TABLE 2.—*Distribution of virus in the internodes of stems, petioles of inoculated leaves, and roots of tomato plants after different intervals following inoculation*

Experiment	Plant No.	Interval in hrs.	Internode												Roots	Petiole		Height of plant in inches
			a	b	c	d	e	f	g	h	i	j	k	l		x	y	
16	1	44	-	-	-	-	-	-	-	-	-	-			-	-	-	14
	2	"	+	+	+	+	+	+	+	+	+				+	+	+	13
	3	"	-	-	-	-	-	+	-	+	-	-			+	-	-	12
	4	"	-	-	-	-	-	-	-	-	-	-			-	-	-	12
	5	"	-	-	-	-	-	-	-	-	-	-			+	-	-	15
	6	"	-	-	-	-	-	-	-	-	-	-	-		-	-	-	17
	7	"	-	-	-	-	-	-	-	-	-	-			-	-	-	13
	8	"	-	-	-	-	-	-	-	-	-	-			-	-	-	14
	9	"	+	+	+	+	+	+	+	+					+	+	+	11
	10	46	-	-	-	-	-	-	-	-	-	-			-	-	-	14
	11	"	-	-	-	-	-	-	-	-	-	-			-	-	-	14
	12	"	-	-	-	-	-	-	-	-	-	-			-	-	-	13
	13	"	+	-	-	-	+	+	+	-	+	+			+	+	-	11
	14	"	-	-	-	+	+	-	-	-	-	-			-	+	+	16
	15	"	-	-	-	-	-	-	-	-	-	-			-	-	-	15
	16	"	-	-	-	+	-	-	-	-	-	-			-	+	+	14
	17	"	-	-	-	-	-	-	-	-	-	-			-	-	-	15
	18	"	+	+	+	+	+	+	+	+	+	+			+	+	+	12
	19	"	-	+	+	+	+	+	+	+	+				-	+	-	10
	20	48	-	-	+	+	+	+	+	+	+	+			+	+	+	14
	21	"	-	-	-	-	-	-	-	-	-	-			-	-	-	15
	22	"	-	-	-	-	-	-	-	-	-	-			-	-	-	15
	23	"	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	16
16 cont'd	24	48	-	-	-	-	-	-	-	-	-	-			-	-	-	13
	25	"	-	-	-	-	-	-	-	-	-	-			-	-	-	14
	26	"	-	-	-	-	-	-	-	-	-				-	-	-	15
	27	"	-	-	-	-	-	-	-	-	-	-			-	-	-	14
	28	"	-	+	+	+	+	+	+	+	+				+	+	+	13
	29	"	-	-	-	-	-	-	-	-	-	-	-		-	-	-	17
	30	50	-	+	+	+	+	+	-	+	+				-	-	-	12
	31	"	-	+	+	+	+	+	+	+	+				-	+	+	11
	32	"	-	-	+	+	+	+	+	-	-	-	-		+	+	-	15
	33	"	-	+	+	+	+	+	+	-	+				+	+	-	10
	34	"	+	+	+	+	+	+	-	-	+				-	+	-	10
	35	"	-	-	-	-	-	-	-	-	-	-			-	-	-	15
	36	"	-	-	+	+	+	+	+	+	+	+			+	+	+	13
	37	"	-	-	+	+	+	-	-	-	+	+			+	+	+	12
	38	"	-	-	-	-	-	-	-	-	-				-	-	-	15
	39	"	-	-	-	+	+	+	+	+	-	+			+	+	-	12
	40	52	-	-	+	+	+	+	+	+	+	+			+	+	+	12
	41	"	-	-	-	-	-	-	-	-	-	-			-	-	-	15
	42	"	+	+	+	+	+	+	+	+	+				+	+	+	12
	43	"	-	-	-	-	-	+	+	+	+	+	+		+	+	-	14
	44	"	-	-	-	-	-	+	-	-	-	-	-		-	+	-	13
	45	"	-	+	+	+	-	+	-	-	-	-			-	+	-	14
	46	"	-	-	-	-	-	-	-	-	-	-			-	-	-	17
	47	"	-	-	+	+	-	+	+	-	+	+	+		-	+	+	13
	48	"	-	+	+	+	-	+	+	+	-	+	-		+	+	+	14
	49	"	-	-	-	-	-	-	-	-	-	-			+	-	-	13
	50	54	-	-	-	-	-	-	-	-	-				-	-	-	12
	51	"	+	+	+	+	+	+	+	-	+	+			+	+	-	13

TABLE 2.—(*Continued*)

Experiment	Plant No.	Interval in hrs.	Internode												Roots	Petiole		Height of plant in inches
			a	b	c	d	e	f	g	h	i	j	k	l		x	y	
16 cont'd	52	54	-	+	+	-	-	-	+	+	+	+	+		+	+	-	12
	53	"	-	-	-	-	-	-	-	-	-	-			-	-	-	12
	54	"	-	-	-	-	-	+	+	+	+	-			+	+	+	12
	55	"	-	-	-	-	+	-	-	-	-	-			-	-	-	14
	56	"	-	-	+	-	-	+	+	+	+	-			+	+	+	13
	57	"	+	+	+	+	+	+	+	+	+				+	+	+	12
	58	"	-	-	-	-	-	-	-	-	-	-			-	-	-	16
	59	"	-	+	+	+	+	+	+	+	-	+	+		+	+	+	14
	60	56	-	-	-	-	-	-	-	-	-	-			-	-	-	13
	61	"	-	-	-	-	-	-	-	-	-	-			-	-	-	13
	62	"	-	+	+	-	+	+	+	+	+	+			-	+	-	15
	63	"	-	-	+	+	-	+	+	+	+	+			+	+	+	13
	64	"	+	+	+	+	+	+	+	+	+	+			-	+	+	13
	65	"	-	-	-	+	+	-	+	+	+	+			-	+	+	15
	66	"	-	-	-	-	-	-	-	-	-	-			-	-	-	16
	67	"	-	+	-	+	+	+	+	+	-	-			+	-	+	15
	68	"	-	-	-	-	-	-	-	-	-	-			-	+	-	15
	69	"	+	+	+	+	+	-	-	-	-	-			-	-	-	15
	70	58	+	-	-	+	+	+	-	-	+	+			+	+	-	14
	71	"	-	+	+	+	+	+	+	+	+	+			+	+	+	15
	72	"	-	-	+	+	+	+	+	+	-	-	-		+	-	+	18
	73	"	-	+	+	+	+	+	+	+	+	+			+	+	+	10
	74	"	-	-	-	-	+	+	-	+	+	-			+	+	+	13
	75	"	-	-	-	-	-	-	-	-	-	-			-	-	-	17
	76	"	-	-	-	-	-	-	-	-	-	-			-	-	-	14
	[illegible]	"	+	+	+	+	-	+	-	+	+	-			+	+	+	14
	78	"	-	+	+	+	+	+	+	+	+	+	-		+	+	+	15
	79	"	-	+	+	+	+	+	-	+	-	-	-		+	+	-	14

60 hours, respectively. Virus had traveled both upward and downward in the stems of 11 plants and only downward in the stems of 10. It had reached the roots of 19 of the plants. In 2 plants (Nos. 18 and 27) in which movement was downward only, it had not reached the roots.

One or more nodal cuttings represented by numbers 1 to 14, beginning with the first section beneath the tip internode, were retained from 15 of the plants in which virus moved. The table shows that many cuttings through which virus passed produced healthy plants. The most striking example of passage through a long section of stem without infecting is presented by plant No. 24, in which the first internodal section below the point of attachment of the inoculated leaf and the roots of the plant were infected but the intervening sections tested were not infected. The piece of stem between and including internodes F and O is estimated to have been at least 10 inches long. None of the 9 nodal or 10 internodal sections into which it was cut were infected.

In plant No. 10, virus was found in the roots and tip at the time sections were tested. The 13 internodal sections cut from the intervening stem proved virus-free. However, 4 of the 12 plants grown from intervening nodal sections became diseased. The piece of stem from which sections B and N and 23 intervening sections were cut is estimated to have been 18

TABLE 3.—*Distribution of virus in nodes and internodes of stems, petioles of inoculated leaves, and roots of tomato plants after different intervals following inoculation*

Experiment	Plant No.	Interval in hrs.	Internodes and nodes[a]																											Roots	Petiole		Height of plant in inches
			a	1	b	2	c	3	d	4	e	5	f	6	g	7	h	8	i	9	j	10	k	11	l	12	m	13	n	14	o		x	y	
17	1	46	-		-		-		+		+		-	+	-	+	-		-		-		-		+							+	+	+	17
	2	"	-		-		-		-		-		-		-		-		-		-		-		-		-					-	-	-	22
	3	"	-		-		-		-		-		-		-		-		-		-		-		-		-		-			-	-	-	21
	4	"	-		-		-		-		-		-		-		-		-		-		-		-							-	-	-	17
	5	48	-		-		+		-		+		-		-		-	+	-		-	-	-	-	-	-	-	-				+	-	+	17
	6	"	-		-		-		-		-		-		-		-		-		-		-		-		-		-			-	-	+	22
	7	"	-		-		-		-		-		-		-		-		-		-		-		-		-		-			-	-	-	21
	8	"	-		-		-		-		-		-		-		-		-		-		-		-		-		-			-	-	-	18
	9	50	-		-		-		+		+		+		+		+		+		+		+		+							+	+	+	18
	10	"	+		-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	+	-			+	-	-	21
	11	"	-		-		-		-		-		-		-		-		-		-		-		-		-		-			-	-	-	21
	12	"	-		-		-		-		-		-		-		-		-		-		-		-		-		-			-	-	-	21
	13	52	-		-		-		+	+	-		+		+		-	+	+		-		-	+	-	-	-	-	-	+		+	+	+	18
	14	"	-		-		+		+		-		-	+	-	+	-	-	-	-	-	+	-	-	-	+	-	-				+	-	+	22
	15	"	-		-		-		-	+	-	-	-	+	-	+	-	+	-	-	-	-	-	+	-	-	-	-	-			+	-	-	19
	16	"	-		-		-		-		-		-		-		-		-		-		-		-		-					-	-	-	19
	17	54	-		-		-		+		-		-		-		-		-		-		-		-		-					+	-	-	17
	18	"	-		-		-		-	+	+		+		-	-	-	-	-	-	-	-	-	-	-	-	-	-				-	-	-	23
	19	"	-		-		-		-	+	-	+	-	+	-		+		-		-		+		+		-					+	-	+	20
	20	"	-		-		-		-		-		-		-		-		-		-		-		-		-					-	-	-	21
	21	56	-		-		+	-	-	+	+		-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-				+	-	-	17
	22	"	+		+		-	-	+		+		-	+	+		+		-	+	+		-	+	+		+		-	+	-	+	-	+	25
	23	"	+		-	+	-	+	+		+		+		+		+		+		-		+		-		-					+	+	+	21
	24	"	-		-		-		+		-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	19
	25	58	+		+		+		+		+		+		+		+		+		+		+									+	+	+	18
	26	"	-		+		-		-	+	+		+		+		+		+		+		+		+		+		+			+	+	+	21
	27	"	-		-		-		-		+		-		-		-		-		-		-		-		-		-		-	-	+	+	20
	28	"	-		-		+		+		-	+	+		+		-	+	+		+		-	+								+	+	+	19
	29	60	-		+		+		+		+		+		+		+		+		-	+	+		+							+	+	+	17
	30	"	-		+		-	+	+		+		+		+		+		+		-	+	+		-	+	+		-	+	+	+	+	+	26
	31	"	+		+		+		+		+		+		+		+		-		-		+		-							+	+	+	21
	32	"	-		-		-		-		-		-		-		-		-		-		-		-		-		-			-	-	-	21

[a] Internodal sections are indicated by letters and plants that grew from nodal sections by numerals.

inches long. Only 4 of the 25 sections into which it was cut were infected. Virus passed through the other 21 sections without infecting.

Uninfected Stem Sections

Samuel assumed that, if virus were present in stem sections incubated in test tubes, it would multiply sufficiently for detection during a period of one week, and that, if it were present in stem cuttings used to produce plants, it would multiply sufficiently for detection in a period of about 6 weeks. However, in attempting to explain why in the experiment presented in his Table II certain test-tube samples gave negative results while all cuttings gave positive results, he states: "It is just possible that in these cases the virus for some reason did not move out from the phloem to the mesophyll tissue."

In my experiments, plants grown from nodal cuttings usually showed mosaic symptoms rather promptly or remained healthy indefinitely, but to this rule there were a few exceptions. For example, the plant grown from internode 12 of plant No. 14 of experiment 17 did not show symptoms of tobacco mosaic until it had reached a height of about 18 inches 61 days after the cutting from which it grew was made. Similarly, the plant grown from internode 8 of plant No. 15 of the same experiment did not show symptoms of tobacco mosaic until it had reached a height of about 14 inches 58 days after the cutting from which it grew was made. It was not believed that disease in these plants could be explained as resulting from accidental infection, since all check plants remained healthy. Results obtained with these two plants and with a few others that showed similar delays in coming down with tobacco mosaic raised the question as to whether or not virus present in the cuttings that produced these plants had remained dormant for considerable portions of the long periods indicated above.

As may be seen from the tables, the likelihood of obtaining virus from sections of tomato stems through which it had passed increased as the interval between time of inoculation and time of cutting was increased. Uninfected sections were never obtained from plants in which systemic symptoms had appeared. This was presumed to result from a continuous flow of virus particles out of primary lesions into stems through which the particles that moved earliest had already passed, but an alternate possibility was that cutting the stems had in some way prevented prompt multiplication of virus present in some or all sections.

As the question of whether or not virus can pass through stem sections without leaving particles behind is an important one, an effort was made to obtain further evidence of presence of virus in plants from cuttings through which, presumably, it had passed without leaving any virus particles. Since it was thought that cutting stems might prevent multiplication of virus present in them, tests were made in which time of cutting was delayed. In order to prevent a continuous flow of virus from inoculated leaflets during the period of delay, the leaflets were cut off 44 hours after inocu-

lations were made. The plants were held under observation until symptoms appeared in the tips of those into which virus had moved. Sections about 1 cm. long were then cut from each internode of the stems of the infected plants. *After* incubation for one week, the sections and roots of the plants were tested. As no uninfected sections or roots were obtained, a different method of limiting the amount of virus that would move out of primary lesions was sought.

It was thought that, if virus passes into and out of long chains of phloem cells without leaving any particles in the cells, it should be possible to delay cutting of plants without contaminating the uninfected regions if a virus were used that would move out of the primary lesions very slowly. The tobacco-mosaic virus strain J14, isolated and described by Jensen (2), never moves from primary lesions in Turkish tobacco plants; but, as Norval (4) has shown, it occasionally produces mutants capable of moving. Therefore, J14 virus was employed in further attempts to study movement in stems. Since this strain is not readily transmitted to tomato plants, Turkish tobacco plants were used.

In an experiment in which 50 young plants were inoculated by rubbing juice containing J14 virus over the upper surface of 4 mature leaves on each plant, all but one of the plants came down with necrotic lesions typical of the disease caused by this virus. The 49 infected plants were set out in deep soil in a greenhouse bench, where they grew vigorously. After a period of about 2 weeks, yellow-mosaic symptoms developed in the tips of 4 of the plants. The viruses causing these mosaics are presumed to have arisen as mutants from J14 virus. The tips of the other 45 plants remained healthy in appearance for some time, but after a period of 2 months necrotic lesions had developed in the upper portions of 10 of the plants, leaving from 1 to 3 feet of healthy-appearing stems bearing healthy-appearing leaves between the primary lesions in the leaves at the bases of the plants and the lesions that developed in the tops. The lesions in the tops were subsequently shown to be caused by viruses similar to J14, but differing from it chiefly in that they were capable of moving out of primary lesions and were somewhat more infectious. These viruses were presumed also to have arisen as mutants from J14. They had to pass through the healthy-appearing stem tissues to reach the tops of the plants. A cutting about 6 inches long was taken from the middle portion of the healthy-appearing section of stem of each of the 10 plants 37 days after secondary lesions appeared in the last plant to show disease in its top.

All of the 10 cuttings rooted and produced plants that remained healthy in appearance for some time. About 6 weeks after the cuttings were started, necrotic lesions developed in 3 of the 10 plants. The lesions were shown to be caused by viruses similar to or identical with those that had passed through the cuttings from which the plants grew. Somewhat later 2 of the 7 remaining plants came down with yellow mosaics. These were presumed to have arisen as mutants from the necrotic-type strains that moved to the tops of the plants from which the cuttings were taken. The other 5 plants, one of which is shown in figure 1, grew to maturity without developing symptoms of disease. In 2 other similar experiments, leaves were removed from several large healthy-appearing plants grown from cuttings through which necrotic-type strains of virus had passed. This was done in the hope that, if virus were present in the phloem of the cuttings, it might be carried to the tips of the plants by the food stream that moves in that direction after defoliation. In no case did disease develop in the defoliated plants. The experiments proved that healthy tobacco plants can be grown from sections of stems through which virus of necrotic-type strains of tobacco mosaic has passed, even when the cuttings from which the plants are grown were made long after virus has moved through the stems.

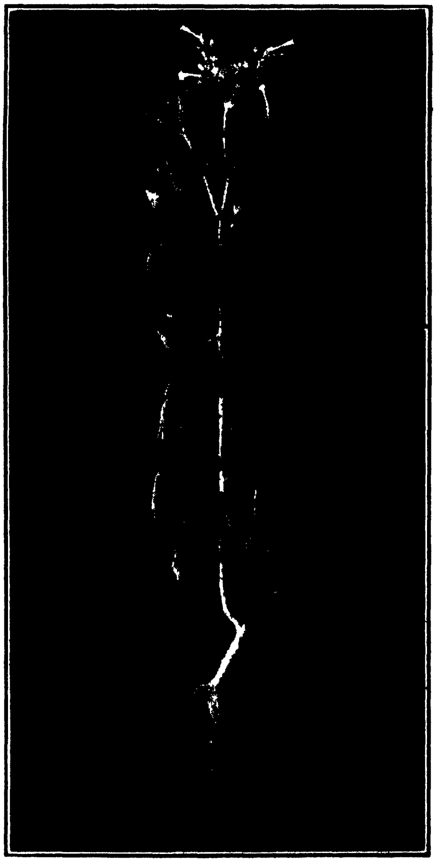

FIG. 1. A healthy Turkish tobacco plant grown from a piece of stem through which a necrotic-type strain of tobacco-mosaic virus had passed. (Photograph by J. A. Carlile.)

The work with tobacco plants confirmed that with tomato plants as regards the ability of virus particles to pass for long distances without infecting. It also confirmed the observation that disease sometimes develops in plants grown from cuttings through which virus has passed, after the plants have remained healthy for a long time. It is evident that virus particles may travel long distances through phloem cells of tomato and tobacco without infecting. This probably results from the movement of virus out of the sections. It is also evident that occasionally virus particles that do not move out of a section may remain dormant in the tissues for a long time before multiplying and causing disease.

In support of the latter conclusion, the writer wishes to record an observation on the behavior of Abutilon mosaic in *Abutilon striatum* Dicks. As is well known from the work of Baur (1), Abutilon plants may be cured of mosaic by carefully cutting out all chlorotic spots that appear in the leaves during a period of several weeks. At least 50 plants cured in this way have been held under observation for long periods of time. Some have been kept as long as 4 years. In only one instance has a plant that was considered cured suffered a relapse. In November, 1936, 20 mosaic-diseased Abutilon plants of the same age, that had been grown from cuttings of a single plant, were divided into two lots of 10 plants each. The plants were observed daily, and all chlorotic spots were cut from the leaves of the first lot. From time to time, areas of similar size were cut from the leaves of the other 10 plants, which served as checks, but care was taken to leave plenty of chlorotic tissue. As the experiment progressed, fewer and fewer spots appeared in the new leaves produced from the plants receiving curative treatment, but the usual number developed in the new leaves of the check plants. Finally, on January 12, 1937, one chlorotic spot was found and removed from a young leaf of one of the treated plants. Following this operation, no more spots developed in the leaves of the treated plants and no further cutting

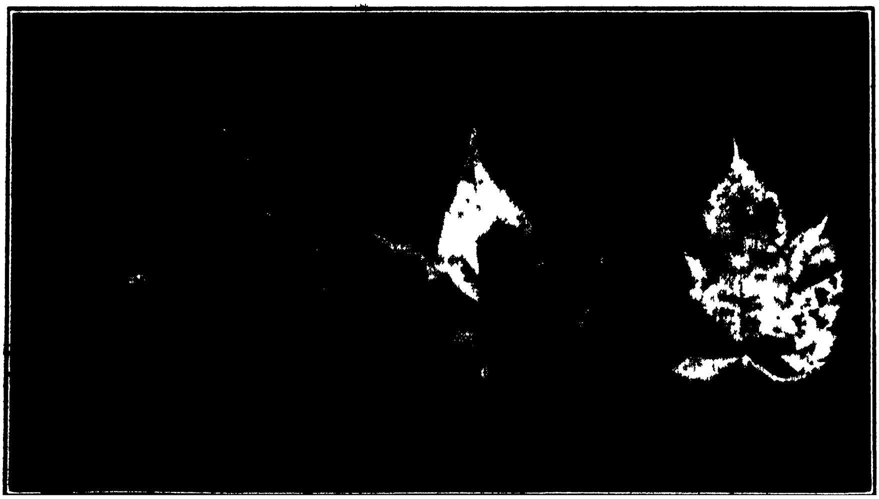

FIG. 2. Leaves of *Abutilon striatum* showing early symptoms of mosaic in a plant that had been cured and had remained healthy in appearance for 7 months. The leaf at the left bearing a single chlorotic spot was the first to show the disease. (Photograph by J. A. Carlile.)

was done. Both check plants and treated plants grew vigorously. The former were typically diseased. The latter appeared healthy and were considered to have been cured. On August 18, 1937, a little more than 7 months after treatment was stopped, a single yellow spot appeared in a leaf at the tip of a branch on one of the plants thought to have been cured. Typical Abutilon mosaic appeared in the leaves that developed above the one bearing the single spot. This leaf and the two borne immediately above it are shown in figure 2. The Abutilon-mosaic virus particle which produced the chlorotic spot shown in the leaf at the left must have remained in a quiescent state somewhere in the plant during a period of more than 7 months. There can be no question of accidental infection in this case, since Abutilon mosaic is not mechanically transmissible except by grafting. After the leaves shown in the photograph were removed on September 15, 1937, the branch was cured by the usual cutting operations. The plant has not suffered a second relapse during the period of about one and one-half years that has passed since it received the second curative treatment.

DISCUSSION

In the experiments described in this paper, it was shown that tobacco-mosaic virus moved out of inoculated leaflets of a considerable number of tomato plants in less than two days. This interval is approximately one day shorter than was reported by Samuel. The difference is presumed to have resulted from a difference in the conditions under which the two sets of experiments were made.

The tables presented show that tobacco-mosaic virus did not move from inoculated leaflets into different plants after even approximately equal periods of time. It was well distributed throughout some plants long before it began to move in others. The irregularity in time of movement was about the same in plants belonging to a clone as in seedlings. Virus began to move in some plants in less than 44 hours, but did not move in others in twice that length of time. Under the conditions obtaining in the experiments described, it never began movement out of inoculated leaflets in 42 hours or less. In 44 hours it moved to all parts of some plants. This evidence that virus may travel throughout a fairly large plant in less than two hours shows the speed with which it is capable of moving through tomato stems. Virus is estimated to have traveled at a rate of at least 7 inches per hour in plant No. 2 of experiment 16. It may have moved at a somewhat faster rate, but is not believed to have greatly exceeded this speed.

The data in the tables also show that, on reaching the stem, virus frequently traveled both upward and downward but also frequently traveled downward only and occasionally upward only. In a considerable number of plants it moved upward in the stems before it reached the roots. It rarely reached the tip of a plant before roots were invaded, but this did sometimes occur. The data show that in a considerable number of plants the virus that moved upward did not travel *via* the roots.

Samuel's discovery that tobacco-mosaic virus may pass through long pieces of tomato stems without infecting them is confirmed. As already stated, virus passed through one piece of stem estimated to have been about 10 inches long without causing infection. It is probable that at least 60 minutes were required for the virus particle or particles to travel this distance. During that time it either did not multiply or gave rise to a progeny that remained dormant in the section or moved out of the section. The former seems the more likely of the two possibilities. It is not to be expected that large numbers of virus particles would move through long chains of phloem cells without some particles becoming lodged in the cells. Since the evidence indicates that tobacco-mosaic virus remains dormant in stem sections only in exceptional cases, it is concluded that virus particles either do not multiply or multiply very slowly as they move through stems.

Because of the speed with which tobacco-mosaic virus moves in stems, it is presumed to travel in sieve tubes, tissues through which food materials are known to move. How either virus particles or metabolites are carried by these tissues is not known. The speed of virus movement and the fact that a particle may apparently travel a long distance in one direction suggest mass flow from cell to cell. The further fact that virus may be carried both upward and downward at the same time indicates the presence of streams moving in both directions simultaneously. This does not mean, of course, that virus travels in both directions simultaneously in the same phloem cells. The movement upward and downward may take place in different strands.

In experiments with both tomato and tobacco, a few plants grown from stem cuttings through which virus had passed without causing infection came down with disease after remaining healthy in appearance for several weeks. This suggests that occasionally virus particles may remain dormant for a long time and may then suddenly begin to multiply. Whether inability of the particles to increase in number during such an interval results from their failure to escape from the phloem is not known. Observations on the development of Abutilon mosaic in an Abutilon plant that had been cured and had remained healthy in appearance for about 7 months suggest that in rare instances particles of this virus also may lie dormant in the plant. A possible explanation of dormancy of virus in the Abutilon plant is not difficult to find. The fact that Abutilon mosaic can be cured by amputation of leaf tissues is strong evidence that the virus causing Abutilon mosaic cannot multiply in stem tissues. If this be true, then it is necessary to postulate only that the virus particle that remained dormant for about 7 months was in stem cells during that period of time. Why it finally passed out into a leaf where it was able to multiply and cause disease and why it did not pass out at a much earlier date are questions that cannot be answered at this time. A similar explanation may apply in cases of dormancy of tobacco-mosaic virus. This virus is known to multiply in tomato and tobacco stems, but it may not multiply in all tissues of the stems The fact that it can pass through long sections without infecting suggests inability to multiply in sieve-tube cells. However, these exceptional cases do not bring into question the conclusion that tobacco-mosaic virus can pass through long pieces of tomato stems without infecting them and that this probably results from movement of all virus out of the pieces.

The movement of tobacco-mosaic virus in tomato plants is very different from that of yellows virus in peach trees. When inoculated by means of budding into a stem about halfway up a tree, the latter virus moves much more rapidly downward than upward (3). Under similar conditions, the rate of movement of yellows virus in peach trees is much slower than that of tobacco-mosaic virus in tomato plants. Whether these differences in direction and rate of movement of yellows and tobacco-mosaic viruses result from differences in the plants they invade or from differences in the viruses is not known. It is possible that the differences noted may reflect differences in the rates and directions of movements in the food streams of the two plants, but it is also possible that they may reflect differences in the virus particles.

SUMMARY AND CONCLUSIONS

Under the conditions of the experiments reported in this paper:

(1) Tobacco-mosaic virus never began movement out of inoculated leaflets of tomato plants in 42 hours or less. It occasionally moved throughout the stem of a plant in 44 hours, but the interval required varied greatly with different individuals.

(2) On first reaching the stem from an inoculated leaflet, the virus usually moved both upward and downward. It sometimes moved downward only, and occasionally upward only. No evidence was obtained in support of the hypothesis that passage of virus from an inoculated leaf to the tip of a plant is *via* the roots. Proof was brought that it did not follow such a course in certain plants.

(3) When movement from an inoculated leaflet begins, virus travels rapidly. It sometimes travels at a rate of 7 inches per hour or faster.

(4) Samuel's observation that in the earliest stages of entering the stem virus particles may be separated by considerable distances is fully confirmed. The particles must pass through long chains of cells without infecting. This movement cannot, therefore, result from propagation by means of an autocatalytic reaction.

(5) Virus particles that have remained for some time in a dormant condition in sections of tomato or tobacco stems may move out into plants grown from such sections and there multiply and cause disease.

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THE FUNGICIDAL ACTIVITY OF PHENOTHIAZINE AND SOME OF ITS OXIDATION DERIVATIVES

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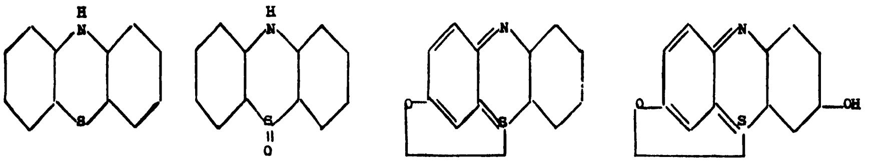
Phenothiazine (thiodiphenylamine) has been known for a number of years (1) and, because of its insecticidal value, has become the subject of a number of researches (2, 3, 4, 8, 9, 10, 13, 14, 15, 16). Gersdorff and Claborn (5) have investigated its ichthyocidal properties. It has received some attention as a fungicide in connection with general laboratory tests (11, 12, 17), but its possibilities as an orchard fungicide apparently have not been investigated.



Phenothiazine Phenothiazine sulphoxide Phenothiazone Thionol

FIG. 1. Structural formulae of phenothiazine and its three oxidation derivatives.

The oxidation derivatives (Fig. 1), phenothiazine-sulphoxide, phenothiazone, and thionol, have been investigated by Gersdorff and Claborn (5) as ichthyocides, and unpublished reports by Siegler and Fink have shown that phenothiazine-sulphoxide and phenothiazone are not effective against codling moth and mosquito larvae. Figure 1 shows the structural arrangement of these compounds.

Following the discovery by Smith, Munger, and Siegler (15) that phenothiazine is insecticidal to codling-moth larvae, routine tests were conducted by the authors to determine its phytocidal properties. In a limited number of tests it was found that phenothiazine may be safely applied to the leaves of apple, pear, grape, rose, lilac, and zinnia, but not to the peach and the common bean (*Phaseolus* sp.). The authors have observed no deleterious effects on apple fruits, but this phase of the problem needs further study.

EXPERIMENTAL PROCEDURE

Since the methods used in this laboratory for conducting fungicidal tests are fully described in previous papers (6, 7), they will be but briefly outlined here. Conidia of *Sclerotinia fructicola* (Wint.) Rehm, obtained from 8-day-old pure cultures growing on sterilized string beans, and *Glomerella cingulata* (Ston.) Spauld. and von Schrenk, obtained from 4-day-old pure cultures grown on potato hard agar, or from apples inoculated with our laboratory strain, are used in the following tests:

The conidia of both species are dispersed in cubes of water agar and perfused by saturated solutions of the given chemical in distilled water for 24 hours. During and after the perfusion tests, observations are recorded as to the degree of toxicity of the soluble materials. Cover slips are sprayed in the laboratory with the test chemical, which may be combined with a carrier. These are transferred to the orchard and suspended from apple trees, where the spray residues are subjected to weathering under orchard conditions. At frequent periods the residue-containing slips are brought into the laboratory and the toxicity of the residue is determined.

In the case of phenothiazine only, field testing was conducted by spraying the chemical at the rate of 2 lb. to 50 gal. of water, with or without a carrier, on apple varieties to control scab, caused by *Venturia inaequalis* (Cke.) Wint.

FUNGICIDAL ACTIVITY OF PHENOTHIAZINE

Phenothiazine in a pure state, according to Bernthsen (1) is a light-yellow crystalline compound melting at 180° C. The compound is easily oxidized in air to a light-greenish compound, and the commercial product now on the market is of that color. Further oxidation, according to Bernthsen (1), results in the formation of a red and even a violet compound. Bernthsen (1) does not comment on the behavior of phenothiazine in water, but it was our experience that the compound is sparingly soluble and difficult to wet in water. A saturated aqueous solution is transparent and colorless. It is insoluble in chloroform. On aging in the presence of water, light, and air, a soluble, pink oxidation derivative is formed. This oxidation change is accelerated by strong outdoor light. If phenothiazine is incorporated in a matrix of hydrated lime and bentonite in water and sprayed on glass cover slips or on plant surfaces, exposure to light and air causes the formation of a purple-red oxidation compound in the dried alkaline residue. This change occurs first in the superficial layers of the exposed residue, but as the exposure is increased the whole mass becomes a deep purple-red.

In our laboratory studies of the fungicidal properties of saturated aqueous solutions of phenothiazine, as shown in table 1, the clear colorless solution was found to be nontoxic. Aging of this solution for 2 weeks in diffuse laboratory light caused the formation of a pink oxidation product that was moderately toxic to the conidia of *Sclerotinia fructicola,* but not to those of *Glomerella cingulata.* At the end of 4 weeks of exposure to the laboratory light and air, enough of the oxidation product was present to be extremely toxic to *S. fructicola.*

Saturated solutions of phenothiazine were exposed to strong outdoor light in ordinary 9-lb. acid bottles. To hasten complete saturation, wetting agents

TABLE 1.—*Fungicidal activity of saturated solutions of phenothiazine*[a] *exposed to ordinary diffused laboratory light*

Exposure to light	Conidial exposure to saturated solution	Effect on *Sclerotinia fructicola* conidia	Subsequent growth[b]	Effect on *Glomerella cingulata* conidia	Subsequent growth[b]	Growth of check conidia[b]	
						Sclerotinia fructicola	*Glomerella cingulata*
	Hours		*Per cent*		*Per cent*	*Per cent*	*Per cent*
2 days. No color to saturated solution	5	No apparent injury	98				
	6	Few budded	96				
	24	do.	96			96	
2 weeks. Saturated solution slightly pink	2	No apparent injury	100	No apparent injury	100		
	4	Few budded	76	18% germinated	100		
	6	do.	78	10% germinated	100		
	24	6% with short tubes	12	42% germinated	100	100	100
4 weeks. Saturated solution of a pink color	3	No apparent injury	98				
	24	All plasmolyzed	0			98	

[a] Ten grams of phenothiazine added to 2000 cc. of distilled H_2O.
[b] Transfers made to potato hard agar and incubated for 24 hours.

TABLE 2.—*Fungicidal activity of saturated solutions of phenothiazine*[a] *exposed 7 days to bright outdoor light*

Wetting agent	Conidial exposure to saturated solutions	Effect on *Sclerotinia fructicola* conidia	Subsequent growth[b]	Effect on *Glomerella cingulata* conidia	Subsequent growth[b]
	Hours		*Per cent*		*Per cent*
Sulfonated aromatic hydrocarbon (deep purple color to solution)	2	Few apparently injured	24	Many appear plasmolyzed	48
	4	All apparently injured	0	All apparently injured	0
	6	All plasmolyed	0	do.	0
	22	do.	0	do.	0
	24	do.	0	do.	0
Soluble fish-oil soap, very opaque, (no color to solution)	2	No apparent injury	100	No apparent injury	100
	4	do.	100	do.	100
	6	do.	96	do.	100
	22	do.	100	do.	100
	24	do.	96	do.	100
None (purple color to solution)	2	Few apparently injured	94	Many appear plasmolyed	78
	4	do.	86	All appear injured	34
	6	Many appear injured	48	do.	26
	22	All appear plasmolyzed	0	do.	0
	24	do.	0	do.	0
Check	6	100% with short germ tubes	100	90% with short germ tubes	100
	24	96% with short germ tubes	100	100% with short germ tubes	100

[a] Ten g. of phenothiazine to 2000 cc. distilled H_2O.

[b] Transferred to potato hard agar, incubated for 24 hr.

were added to the water and the suspensions were allowed to settle. The higher sulfonated alcohols, when used as wetting agents, yielded clear solutions, while fish-oil soap brought about a suspension that did not settle. When the solution was clear, as with no wetting agents or with the sulfonated alcohol, the irradiation developed a deeply purple-red. When the suspension was present, no color appeared.

The results of studies with the above solutions are recorded in table 2. The deep purple-red solution, obtained from the strongly irradiated phenothiazine, wetted by sulfonated alcohol, was the most toxic to both species, while that feebly wetted, but of a more dilute purple-red, was less toxic. The solution obtained by wetting with the soluble fish-oil soap was not toxic to either species. The lack of development of the toxic oxidation derivative in this case probably was due to lack of light penetration into or through the colloidal suspension. In some cases the toxic solutions were filtered through L3 Chamberlain-Pasteur candles, and the filtrates, which were a deep-red, were evaporated to dryness at 50° C., or more. During the drying process a reddish constituent of the mixture was sublimed on the paper towels on which the evaporating dishes were placed. In some cases filtered and nonfiltered toxic solutions were extracted with chloroform, and a major portion of the constituents, reddish in color, of the mother filtrate, was recovered in the chloroform extract. Solutions from which the chloroform soluble constituent was removed were nontoxic, while water solutions prepared from the chloroform extracts, after removal of the chloroform, were toxic.

Many tests were conducted with phenothiazine-lime-bentonite residues exposed to various periods of weathering. Tables 3 and 4 present the details of some of these experiments. Table 3 shows quite clearly that exposure to outdoor weathering (irradiation) is necessary before toxicity becomes apparent.

Toxicity developed rapidly with the residues exposed in the orchard, but none developed with the residues stored in the laboratory. The residues collected from the field, after a period of 11 days' exposure to light, air, and rainfall, still possessed toxic properties. Those collected after 14 days, during a rainstorm, appeared to have been partly depleted, while those taken after 21 days appeared to be entirely depleted of their toxic oxidation products.

Table 4 shows the fungicidal activity of a phenothiazine mixture as compared with Bordeaux mixture and half-strength copper phosphate mixture residues. Bordeaux mixture is a strong fungicide, whereas copper phosphate mixture is a weak fungicide. In all of our tests the phenothiazine mixture residues were found to possess excellent fungicidal activity when exposed to weathering. This activity compared favorably with that established by Bordeaux mixture under the same environmental conditions. The results show, that if the residue persists, weathering alters the fungicidal value of phenothiazine mixture less rapidly than it does that of Bordeaux mixture. This suggests that the toxic principle of the phenothiazine system

TABLE 3.—*Effect of outdoor and indoor weathering on the fungicidal activity of residues containing phenothiazine,[a] lime, bentonite and wetting agent*

Treatment	Days weathered	Precipitation totaled	Effect on *Sclerotinia fructicola* conidia[d]	Subsequent growth[b]	Effect on *Glomerella cingulata* conidia[d]	Subsequent growth[b]	Check growth[c]	
							Sclerotinia fructicola	*Glomerella cingulata*
		Inches					*Per cent*	*Per cent*
Weathered in outdoor atmosphere. Slips hung up in apple trees.	7	0.00	All plasmolyzed	0	All plasmolyzed	0	100	100
	10	0.00	do.	Few	do.	0	92	100
	11	0.23	Mostly all appear plasmolyzed	do.	Many appear plasmolyzed	Few	74	100
	14	0.71	Some appear plasmolyzed	do.	Many with buds	Many	74	100
	27	1.61	No apparent injury	Many	No germination. All normal	do.	100	100
Weathered in laboratory atmosphere. Slips stored in slide box.	1	0	No apparent injury	Many	All germinated	Many	68	100
	3	0	do.	do.	Many germinated (62%)	do.	92	100
	11	0	Few germinated. No injury	do.	All germinated	do.	100	100
	15	0	No germination. All normal	do.	do.	do.	92	100
	16	0	do.	do.	do.	do.	74	100
	29	0	Few germinated. All normal	do.	do.	do.	100	100

[a] Phenothiazine 2 lb., lime (hydrated) 4 lb., bentonite 2 lb., water 50 gal., wetting agent ¼ oz. sulphonated alcohol.
[b] Transferred to potato agar and incubated for 24 hours before observations were made.
[c] Percentage of growth of conidia at the end of a 24-hour incubation period in van Tieghem cells.
[d] Observations made at end of 24-hour incubation period in van Tieghem cells.

TABLE 4.—*Effect of weathering on the fungicidal activity of residues containing phenothiazine as compared with Bordeaux mixture and copper phosphate mixture*

Combination	Days weathered[a]	Precipitation totaled	Effect on *Sclerotinia fructicola* conidia[d]	Subsequent growth[b]	Effect on *Glomerella cingulata* conidia[d]	Subsequent growth[b]	Check growth[c] *Sclerotinia fructicola*	Check growth[c] *Glomerella cingulata*
		Inches					*Per cent*	*Per cent*
Phenothiazine 2 lb.; hydrated lime 4 lb.; bentonite 2 lb.; sulphonated hydrocarbon ½ oz.; water 50 gal.	3	0.29	All appear plasmolyzed	0	None appear injured	Many	100	100
	5	0.29	do.	0	All appear plasmolyzed	Few	84	100
	8	0.81	do.	0	do.	0	98	100
	15	0.81	do.	0	do.	0	68	92
	22	0.88	do.	0	do.	Few	100	35
	27	1.11	do.	0	Few germinated. Mostly plasmolyzed	do.	Few	96
	29	2.67	Light residue. Mostly all plasmolyzed Some not injured	Few	Heavy residue. All appear plasmolyzed	0	62	100
Copper sulphate 2 lb.; hydrated lime 4 lb.; water 50 gal.	8	1.01	All appear plasmolyzed	0	All appear plasmolyzed	0	100	100
	12	1.01	do.	Few	do.	Few	84	100
	13	1.53	do.	0	do.	Few	98	100
	20	1.53	do.	Few	do.	0	68	92
	25	1.60	do.	0	do.	0	96	100
	27	1.60	do.	0	do.	Few	100	35
	32	1.83	do.	0	do.	Many	Few	96
	34	3.29	do.	0	Few injured. Many not	do.	62	100
Copper phosphate 1 lb.; hydrated lime 2 lb.; bentonite 1 lb.; water 50 gal	3	0.29	Many appear injured	0	All appear normal	Many	100	100
	5	0.29	12% germinated	Many	100% germinated	do.	84	100
	8	0.81	40% germinated. None injured	do.	80% germinated. None injured	do.	98	100
	15	0.81	None appear injured	do.	No germination	do.	68	92
	22	0.88	Some plasmolyzed	Few	Few budded. Few injured	do.	100	35
	27	1.11	Few germinated. Few plasmolyzed	Many	Few germinated. Mostly normal	do.	Few	96
	29	2.67	do.	do.	Many plasmolyzed	do.	62	100

[a] Days slips hung in trees.
[b] Transfers made to potato agar and incubated for 24 hours.
[c] Percentage of growth of conidia at the end of a 24 hour incubation period in van Tieghem cells.
[d] Observations made at end of 24-hour incubation period in van Tieghem cells.

is restored more rapidly than that of Bordeaux mixture. With the copper phosphate mixture residues the toxic principle (soluble copper) is not built up to any great degree (6). Its depletion by rainfall and absorption by conidia probably accounts for the varying results listed in table 4.

During 1937 and 1938, orchard tests with phenothiazine were conducted *on a small scale at the U. S. Horticultural Station at Beltsville, Maryland.* In 1937, phenothiazine, wetted by soluble fish-oil soap, was used at the rate of 2 lb. per 50 gal. of water. Residues built up from such applications were scanty. In 1938 more body was added to the mixture by combining phenothiazine with lime and bentonite at the rate 2-4-2 lb., respectively, to 50 gal. of water. This mixture also was wetted by using soluble fish-oil soap at the rate of ½ lb. to 50 gal. of water. On weathering, this residue became purplish pink.

Table 5 shows the results of these tests and also tests of phenothiazine applied in an apple orchard near Vincennes, Indiana. These data were collected by Leslie Pierce, Bureau of Plant Industry, from plots originally designed by L. F. Steiner, Bureau of Entomology and Plant Quarantine, for insecticidal purposes. It was observed and recorded, in detail, at the end of the tests that very little apple scab developed in those plots sprayed with phenothiazine.

The results listed in tables 3, 4, and 5 indicate that irradiated phenothiazine mixture is toxic to the organisms that cause peach brown rot, apple bitter rot, and apple scab. The results of the perfusion tests showed that the toxicity of phenothiazine mixture was attributable to the formation of some compound from phenothiazine in the presence of light and air and that this compound, when dissolved in water, is purplish red and is soluble in chloroform. It sublimes when dried at 50° C. or more. These observations led us to examine the fungicidal properties of the known oxidation derivatives of phenothiazine in order to determine those toxic to fungus conidia.

THE FUNGICIDAL ACTIVITY OF PHENOTHIAZINE SULPHOXIDE, PHENOTHIAZONE, AND THIONOL

Through the courtesy of R. C. Roark and associates, Bureau of Entomology and Plant Quarantine, samples of phenothiazine sulphoxide and phenothiazone were secured from that bureau. From Floyd De Eds, Bureau of Chemistry and Soils, stationed at San Francisco, California, a sample of thionol and directions for its production (4) were obtained. Other samples of phenothiazone and thionol were prepared by the junior author and applied in some of the tests.

Phenothiazine sulphoxide is a light-yellow crystalline compound, sparingly soluble in water and in chloroform, and has a melting point of 250° C. Saturated aqueous solutions, at first colorless, change to a feeble pink when exposed to weak light and to a deep purple-red when exposed to strong light in the presence of air. When the deeply purple-red saturated solution is filtered through an L3 Chamberlain-Pasteur candle, 2 fractions are secured.

TABLE 5.—*Effect of phenothiazine sprays on apple scab and russet*

Year	Treatment	Variety	Number examined	Number russeted	Percentage russeted	Number scabbed	Percentage scabbed
1937 5 applications	Phenothiazine 2–50	Williams	334	40	11.9	2	0.7
	do.	York Imperial	29	0	0.0	0	0.0
	do.	Starking Delicious	78	9	11.5	1	1.3
	(Lime-sulphur 1–50, 4 applications) (Copper phosphate 2–50, 1 application)	do.	759	57	7.5	0	0.0
	Check	do.	96	0	0.0	41	42.7
1938 **6 applications**	Phenothiazine, lime, bentonite[a]	Oldenburg	675	0	0.0	0	0.0
	do.	Rome	21	0	0.0	0	0.0
	do.	Starking Delicious	10	0	0.0	0	0.0
	Bordow 4–50	Oldenburg	546	427	78.2	0	0.0
	Lime-sulphur 1–50	do.	31	0	0.0	0	0.0
	Check	Starking Delicious	361	0	0.0	258	71.5
1938 Dyer Orchard, Vincennes, Indiana[b]	Lime-sulphur 1–50, preblossom; wettable sulphur 3–50, calyx; phenothiazine 4–100, 13 cover applications	Grimes	800			40	5.0
	Lime-sulphur 1–50, preblossom; wettable sulphur 3–50, calyx; phenothiazine 4–100, 8 cover applications	do.	800			54	6.7
	Lime-sulphur 1–50, preblossom; wettable sulphur 3–50, calyx; 8–10 cover sprays, fixed nicotine	do.	1600			1401	87.5

[a] Phenothiazine 2 lb., hydrated lime 4 lb., bentonite 2 lb., water 50 gal., wetting agent (⅛ lb. soluble fish-oil soap).
[b] Courtesy of L. F. Steiner, Bureau of Entomology and Plant Quarantine.

One of these is a deep-purple, very insoluble material, which, when suspended, imparts a feeble purple tint to distilled water. The other fraction is soluble and imparts a deep-red to distilled water; it is completely soluble in chloroform, and when dried at 50° C. or more, sublimes readily.

The reddish compound associated with the toxicity of the phenothiazine and phenothiazine sulphoxide saturated solutions was, because of its similarity of properties, thought to be phenothiazone. Acquiring sufficient quantities of purified residues of this reddish material from these solutions, for the purpose of melting-point identification, presented an enormous task. The absorption spectrum, therefore, was used for identification through the help and kindness of R. E. Davis, Bureau of Animal Industry. Authentic phenothiazone, prepared by chemists of the Bureau of Entomology and Plant Quarantine, and a chloroform extract of an irradiated filtrate of phenothiazine sulphoxide were used in these tests. Both were known to be toxic to the conidia of *Sclerotinia fructicola* and *Glomerella cingulata.* The results, according to Davis, showed that "while there was some distortion of the curve, as if an impurity was present, the principal bands of absorption and transmission [of the two materials] were in the same places—and the substances were presumably the same." Since the two samples possess almost the same physical and spectrum-absorption properties, they appear to be composed principally of the same compound, i.e., phenothiazone.

Phenothiazone is a dark brownish-red crystalline compound, very soluble in chloroform and in water to about 40 p. p. m., and melting at 162° C. At lower temperatures sublimation occurs, and deep-red colors are imparted to water or solid materials that come in contact with it. The saturated solution in distilled water is clear and a deep purple-red. It resembles in all its characteristics the soluble fraction obtained from the strongly irradiated solution of phenothiazine sulphoxide and phenothiazine. Irradiation of phenothiazone does not cause any change in any of its properties.

Thionol, as furnished to us by Floyd De Eds, is a dark purplish compound and is soluble in chloroform and to a slight degree in distilled water. It does not melt sharply. The saturated distilled-water solution is clear and slightly purple. Irradiation of the clear saturated solution failed to change any of its properties. Thionol, made according to the method of Bernthsen (1), is insoluble in chloroform and only slightly soluble in distilled water, and is of a blue-purple color.

Table 6 shows the effect of saturated water solutions of phenothiazine sulphoxide (irradiated with weak and strong light), phenothiazone, and thionol (DeEds and Bernthsen) on the growth of *Sclerotinia fructicola* and *Glomerella cingulata* conidia. Also included in table 6 are data showing the effects of the 2 fractions isolated from the saturated, strongly irradiated solution of phenothiazine sulphoxide. The soluble deep purple-red fraction was toxic, while the insoluble fraction was not. The saturated solutions of unchanged phenothiazine sulphoxide and thionol possessed little or no toxic properties. Phenothiazone was found to possess a marked degree of toxicity

TABLE 6.—*Fungicidal activity of saturated solutions of phenothiazine sulphoxide, phenothiazone, and thionol*

Treatment	Conidial exposure to perfusion	Effect on *Sclerotinia fructicola* conidia	Subsequent growth[a]	Effect on *Glomerella cingulata* conidia	Subsequent growth[a]	Check[b]	
						Sclerotinia fructicola	*Glomerella cingulata*
	Hours		*Per cent*		*Per cent*	*Per cent*	*Per cent*
Phenothiazine sulphoxide saturated solution. Light pink solution (Indoor)	6	No apparent injury. No germination	88	51% germinated	100	100	100
	24	do.	72	46% germinated	100	100	100
Phenothiazine sulphoxide saturated solution. Deep purple (Outdoor)	6	Some appear injured	52	Many appear plasmolyzed	48	100	100
	24	All appear plasmolyzed	0	All appear plasmolyzed	0	100	100
Filtrate from outdoor irradiated phenothiazine-sulphoxide. Deep red	6	All appear plasmolyzed	0	All appear plasmolyzed	0	100	100
	24	do.	0	do.	0	100	100
Precipitate from outdoor irradiated phenothiazine sulphoxide. Purple	6	Few germinated. No apparent injury	96	75% germinated	100	100	100
	24	16% germinated. No apparent injury	100	78% germinated	100	100	100
Phenothiazone saturated solution. Deep red	6	Many appear injured	12	Many appear plasmolyzed	21	100	100
	24	All plasmolyzed	0	All plasmolyzed	0	100	100
Thionol saturated solution. Purple DeEds sample	6	No apparent injury	96	Few germinated	100	100	100
	24	Many budded. No injury	100	100% germinated	100	100	100
Thionol saturated solution. Purple Bernthsen sample	4	32% with short tubes no apparent injury	100	46% medium germ tubes, no apparent injury	100	100	100
	24	64% germinated, no apparent injury	100	38% medium germ tubes, no apparent injury	100	100	100

[a] Transfers made to potato hard agar and incubated for 24 hours.

[b] Counts made at end of 24-hour incubation period after transfer to potato agar.

TABLE 7.—*Fungicidal activity of various concentrations of phenothiazone in solution*

Concentration	Conidial exposure to perfusion	Effect on *Sclerotinia fructicola* conidia	Subsequent growth[a]	Effect on *Glomerella cingulata* conidia	Subsequent growth[a]	Check germination[b]	
						Sclerotinia fructicola	*Glomerella cingulata*
	Hours		*Per cent*		*Per cent*	*Per cent*	*Per cent*
2.5 parts per million by volume	6	Mostly all plasmolyzed	12	No apparent injury	100	100	100
	22	All appear plasmolyzed	0	78% with short germ tubes	96	100	100
	24	All plasmolyzed	0	65% germinated. Some injured	74	100	100
5.0 parts per million by volume	2	No germination. No apparent injury	86	Few appear injured. No germination	92	100	100
	22	All appear plasmolyzed	0	All appear plasmolyzed	few	100	100
	24	do.	0	do.	0	100	100
40.0 parts per million by volume	6	Apparent injury	8	All appear injured	0	100	100
	22	All appear plasmolyzed	0	All appear plasmolyzed	0	100	100
	24	do.	0	do.	0	100	100

[a] Transfers made to potato agar and incubated for 24 hours before observation.
[b] Counts at end of 24-hour incubation period after transfer to potato agar.

TABLE 8.—*Effect of weathering on the fungicidal activities of residues of lime-bentonite containing phenothiazine sulphoxide and thionol*

Combination	Days weathered[a]	Precipitation totaled	Effect on *Sclerotinia fructicola* conidia[d]	Subsequent growth[b]	Effect on *Glomerella cingulata* conidia[d]	Subsequent growth[b]	Check germination[c]	
							Sclerotinia fructicola	*Glomerella cingulata*
		Inches		*Per cent*		*Per cent*	*Per cent*	*Per cent*
Phenothiazine sulphoxide 2 lb. Hydrated lime 4 lb. Bentonite 2 lb. Water 50 gal.	0	0.00	All appear plasmolyzed	None	All germinated	Many	100	100
	3	0.40	No apparent injury	Many	100% germinated	do.	100	100
	6	0.40	Many app. plasmolyzed	Few	do.	do.	100	100
	13	1.10	Many appear injured	do.	do.	do.	100	100
Thionol 2 lb. Hydrated lime 4 lb. Bentonite 2 lb. Rosin residue emulsion ½ pt. Sulphonated alcohol ¼ oz. Water 50 gal.	5	0.45	No apparent injury	Many	Many germinated	Many	100	100
	7	0.45	All appear normal	do.	All normal. No germ.	do.	100	100
	14	0.45	None appear injured	do.	All appear plasmolyzed	None	100	100
	18	0.45	No germination. No injury	do.	do.	do.	100	100
	21	0.45	All appear normal	do.	do.	do.	92	100

[a] Exposed to orchard environment.
[b] Transfers made to potato agar and incubated for 24 hours.
[c] Counts at end of 24-hour incubation period after transfer to potato agar.
[d] Observations made at end of 24-hour incubation period in van Tieghem cells.

TABLE 9.—*Effect of weathering on the fungicidal activities of lime-bentonite*[a] *residues*[a] *containing varying dilutions of phenothiazone*

Days weathered	Total precipitation	Organism	Toxic effect of dilution of phenothiazone[b]					Check germination[c]
			1/8 lb.	1/16 lb.	1/32 lb.	1/64 lb.	1/128 lb.	
								Per cent
0	0.00	*Sclerotinia fructicola*	Complete	Complete	Complete	Complete	Complete	68
		Glomerella cingulata	do.	do.	do.	do.	Few escape	100
1	0.40	*Sclerotinia fructicola*	do.	do.	do.	do.	do. do.	48
		Glomerella cingulata	do.	do.	Few escape	Many escape	Many escape	100
4	0.40	*Sclerotinia fructicola*	do.	do.	Complete	Complete	Few escape	72
		Glomerella cingulata	do.	do.	Few escape	Few escape	Many escape	100
7	0.40	*Sclerotinia fructicola*	do.	do.	Complete	Complete	Few escape	62
		Glomerella cingulata	do.	do.	Few escape	Many escape	Many escape	76
20	1.10	*Sclerotinia fructicola*	do.	do.	do. do.	Few escape	Few escape	76
		Glomerella cingulata	do.	Few escape	do. do.	do. do.	Many escape	98

[a] Hydrated lime 4 lb., bentonite 2 lb. with indicated weights of phenothiazone in 50 gal. of water.
[b] Growth observed after transfer to potato hard agar following a 24-hour incubation in van Tieghem cells.
[c] Germination at end of 24-hour van Tieghem incubation.

to the conidia of *S. fructicola* and *G. cingulata*. Table 7 shows the effect of very dilute concentrations of phenothiazone on these organisms.

Residues containing phenothiazine sulphoxide, phenothiazone, and thionol combined with hydrated lime and bentonite were prepared and subjected to weathering in the orchard. A ratio of 2 lb. of organic material, 4 lb. of hydrated lime, and 2 lb. of bentonite to 50 gal. of water was used. Tables 8 and 9 show the results of some of these tests. On aging, the residues containing phenothiazine sulphoxide became toxic to the conidia of *Sclerotinia fructicola* but not to those of *Glomerella cingulata*. The effective residues of phenothiazine sulphoxide became faintly pink. Residues of thionol (De Eds) were not toxic to the conidia of *S. fructicola* or *G. cingulata*, but, on aging, became toxic to those of *G. cingulata*. This suggests that De Eds' thionol is oxidized further to some other material, but, so far, we have not succeeded in identifying this compound. Residues containing phenothiazone were found to be toxic to the conidia of both species of fungi, and further tests, as shown in table 9, revealed its potency in residues when used as low as 1/32 lb. in 50 gal. of spray mixture.

No orchard spraying tests against apple scab were conducted with phenothiazine sulphoxide, phenothiazone, or thionol.

Limited phytocidal tests on Carman and Elberta peach varieties were conducted with phenothiazine sulphoxide, phenothiazone, and De Eds thionol. None of these materials caused injury to the leaves or bark. The effect on peach fruits was not recorded, because the fruit harvest was over at the time the tests were conducted. Phenothiazone was not phytocidal to lilac or bean plants.

DISCUSSION

An analysis of the results of various perfusion, residue, and orchard-spraying experiments shows that phenothiazine and its known oxidation derivatives are fungicidally an interesting group.

The data indicate that phenothiazone is the most important member of this group as a fungicide. It is effective at very low concentrations and appears to resist weathering remarkably well. It appears to be the most stable of the oxidation derivatives of phenothiazine. Its solubility in water is of such a degree that a residue containing phenothiazone should furnish a continuous supply of the toxic material over a period of time. This is indicated from the results of the residue tests. Its extreme activity as a fungicide should allow its use at very low concentrations; the cost, therefore, should not be prohibitive. Its usefulness will be increased because of its slight volatility at ordinary outdoor temperatures, which will finally cause it to disappear from the surfaces on which it has been sprayed. Since phenothiazone, from observations made in a limited number of cases, appears noninjurious to the peach, lilac, and bean, it may be widely useful as a plant protectant.

Phenothiazine, since it is in commercial production and known to the trade as an insecticide, is also a very important member of this group.

While it is not directly fungicidal, our experiments indicate that is is readily oxidized to a material that is so. Since phenithiazone in the chemical laboratory is made by oxidation of phenothiazine, it is believed also to evolve in the natural oxidation of solutions and residues containing phenothiazine. This has been verified by *chloroform solubility, sublimation* tests, and spectrum absorption and transmission tests. The continued fungicidal activity over a long period of time in the orchard, as shown by our data on residues containing phenothiazine, is probably caused by the slow natural oxidation of the phenothiazine to phenothiazone as the layers of the residue are exposed by weathering.

Phenothiazine sulphoxide is an oxidation product of phenothiazine. Saturated solutions of this material appear to form, in the presence of light and air, oxidation products similiar in their activity to phenothiazone and thionol. When oxidized and combined with hydrated lime and bentonite in a residue it is not so active a fungicide as phenothiazine. It appears, therefore, less useful as a fungicide than phenothiazine and phenothiazone. Because it is unstable in solutions, in the presence of light and air, it has become useful for isolating phenothiazone in a pure state.

Thionol appears to be the most uncertain member of this group from both a chemical and a fungicidal standpoint. The material in our possession has no fungicidal properties when dissolved directly in water, yet residues containing De Eds' thionol, when subjected to weathering (oxidation), become toxic to the conidia of *Glomerella cingulata.* Since phenothiazine and phenothiazone exert a fungicidal action against the conidia of *G. cingulata* as well as other fungi, it is extremely doubtful that thionol would be equal to these substances in disease control.

SUMMARY

A study was made of the fungicidal efficiency of phenothiazine and its oxidation derivatives phenothiazine sulphoxide, phenothiazone, and thionol.

The toxicity studies consisted of subjecting conidia of *Sclerotinia fructicola* (Wint.) Rehm. and *Glomerella cingulata* (Ston.) Spauld. and von Schrenk to perfusion by saturated solutions and to weathered and nonweathered residues containing the organic materials.

Toxicity studies were also conducted with phenothiazine in field spraying tests against the conidia of *Venturia inaequalis* (Cke.) Wint. on apple varieties.

Phenothiazone proved to be the most efficient fungicide of the group. This material, in aqueous solution, was found to be toxic at several parts per million to the conidia of *Sclerotinia fructicola* and *Glomerella cingulata.*

Phenothiazone was shown, by chloroform solubility, sublimation and spectrum absorption and transmission tests, to be the toxic product formed when phenothiazine and phenothiazine sulphoxide were oxidized in the presence of air, light, and water.

Phenothiazine residues and saturated aqueous solutions, when unaltered by oxidation, proved to be nontoxic to the conidia of both of these species.

Phenothiazine, when altered by oxidation, was found to be toxic to the conidia of *Sclerotinia fructicola, Glomerella cingulata,* and *Venturia inaequalis.*

Phenothiazine sulphoxide, when exposed to oxidation in alkaline residues, was toxic to the conidia of *Sclerotinia fructicola* but not to the conidia of *Glomerella cingulata.*

Saturated aqueous solutions of phenothiazine sulphoxide, after being oxidized by strong light and in the presence of air, proved to be toxic to the conidia of *Sclerotinia fructicola* and *Glomerella cingulata.*

Saturated aqueous solutions of thionol were not toxic to the conidia of *Sclerotinia fructicola* or *Glomerella cingulata.*

Thionol residues, on oxidation, became toxic to the conidia of *Glomerella cingulata,* but not to the conidia of *Sclerotinia fructicola.*

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INOCULATION EXPERIMENTS WITH LOOSE SMUTS OF WHEAT AND BARLEY (USTILAGO TRITICI AND U. NUDA)[1]

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INTRODUCTION

Whereas, for many plant diseases suitable inoculation methods have been established, there are still some for which these methods are not in all respects satisfactory. Thus, for the inoculation of wheat and barley with loose smut, *Ustilago tritici* (Pers.) Rostrup and *Ustilago nuda* (Jens.) K. and S., a number of methods have been described each of which has its advantages and disadvantages, but it cannot be said that *the* standard method has yet been found.

In the Institut für Pflanzenzüchtung, Halle (Germany), which for many years has been working on the problem of loose smut, use is very often made of the *"Einzelblütenspritze"* (indicated below as the Halle method), with which each individual flower is inoculated with dry spores (Roemer, Fuchs, and Isenbeck, (3)). The drawback with this as well as other methods is that the setting and viability of the smut-infected seed and the winter-hardiness of the plants grown from such seed may suffer to such extent as to result in losses amounting to 20–50 per cent; in case of winter barley, according to Thren (4), even more. Pech's method (2), likewise of German origin, in which the entire head is treated *in vacuo* with dry spores, seems little used.

In 1936, Moore (1) described an apparatus enabling one to inoculate with a spore suspension entire heads *in vacuo.* The results thus obtained are very good. No data, however, are given concerning the most favorable period for inoculation, or the optimum concentration of spores and the requisite number of pump strokes.

It, therefore, seemed desirable to investigate more closely the different factors influencing the degree of success of inoculation according to Moore's method. Owing to lack of time and help no comparison could be made between Moore's and the Halle methods. The aim of the investigation here reported is not to establish the superiority of the one method to the other, but only to show that Moore's method with some slight modifications yields very good results and enables us without special training to perform a larger number of inoculations per hour than is possible with the Halle method with well-trained help.

METHODS AND MATERIALS

The apparatus used for the inoculations (Fig. 1) has been adequately described by Moore (1), so that here a short repetition will suffice in which especially some modifications will be brought forward. The apparatus comprises a supporting rod (A), a flask for the spore suspension (B), an inoculating chamber (C) and a hand-operated vacuum pump (D). The lower portion of the rod is made of iron, to which an iron step is attached to push the rod into the ground. The clamp used by Moore to support the inoculum flask was substituted by a wire-net basket in order to prevent the breaking of the flask. The upper portion of the rod is made of wood and equipped with brass hooks on which by means of a spring the inoculating chamber may be suspended at each desired height. The inoculating chamber consists of a large glass tube with a narrow neck. The tube may be closed tightly with a rubber stopper Through a hole in the stopper the tube is connected by means of a rubber tube with the inoculum flask. Through a slit in this stopper the heads that are to be inoculated can be inserted in the inoculating chamber. At the top the chamber is connected with the pump by means of a vacuum-rubber tube. In this rubber tube a T-tube with a stopcock is inserted in order to admit the air after the in-

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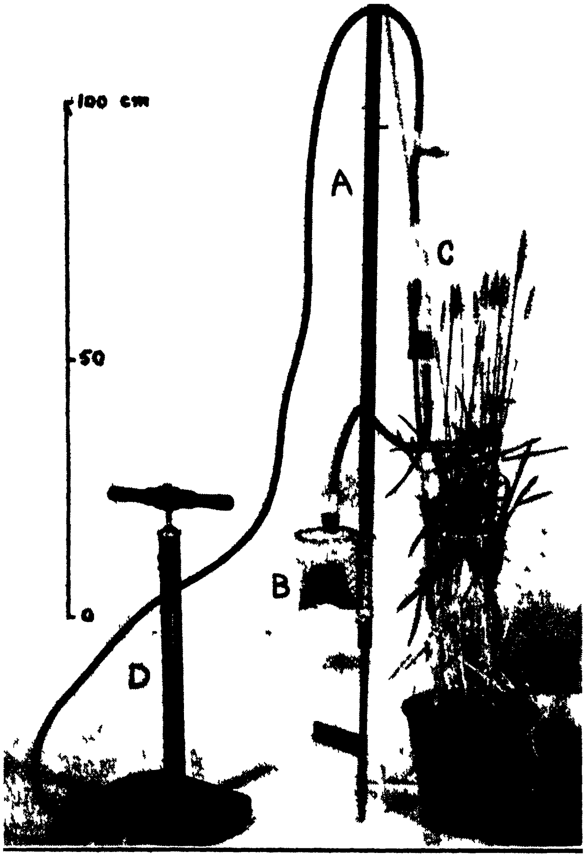

FIG 1. Moore's apparatus as modified by the author. For explanation, see text.

oculation. Inasmuch as the rubber tube, owing to its weight, would cause the inoculating chamber to hang in an oblique position, it is laid across one of the hooks of the rod. A large bicycle pump with an inverted leather plunger makes a satisfactory vacuum pump. Contrary to Moore's method, no vent holes were bored in the upper part of the pump, so that the vacuum virtually remains after each stroke.

The inoculation is performed as follows. The flask (contents ½ l.) is filled with an opaque brownish-black suspension of spores. For 300–400 cc. of liquid six smutted heads suffice. The concentration is then so high that it equals the concentration 1 (see below) or even surpasses it. Through the slit of the rubber stopper a number of haulms are inserted just below the head. A mixture of wax and vaseline is added to render the stopper airtight.[2] The heads are now covered by the tube (C), which, with the aid of the spring and the vacuum-rubber tube, is suspended at the desired height. The spore suspension is now carefully drawn up into the inoculating chamber until it has risen above the heads and consequently fills most of the tube. Then the connection between the flask and the inoculating chamber is broken by pinching the connecting tube with the fingers (in practice more convenient than with a Mohr pinchcock). A number of strokes is made with the pump so as to replace the air between the glumes with the spore suspension. The outer air is then admitted through the stopcock and the connection between glass tube and flask is restored, so that the liquid flows back into the flask. The heads are then taken out of the glass tube, the remainder of the wax-mixture is removed from the haulms.

After some experiments it was easily possible to inoculate 4 heads at a time. In general, it is rather easy to find 4 haulms equal in length and development. Thus, a considerable increase is obtained in the number of inoculated heads per hour. In 1938, the second year of our experiments, two persons could thus easily inoculate 80 heads of wheat per hour. With barley, owing to the weakness of the haulms, especially with 2-rowed spring barley, no more than about 50 heads per hour could be selected, inoculated, and marked. In contrast to Moore's method with barley, the sheath of the upper leaf was carefully unfolded and turned down. In most cases the treatment was then successful without breaking the haulms. In the case of barley, the heads were inoculated as soon as they had fully emerged from the boot. The inoculation period for wheat, however, was from the commencement to the middle of anthesis. The number of pump strokes was 10 for wheat and 20 for barley. The velocity of pumping is of no importance, because the wax-vaseline mixture prevents almost all leakage along the stem.

The experiments were planned with the intention to establish: (a) The optimum period for inoculation; (b) the optimum concentration of spores; (c) the optimum number of pump strokes. They were performed with *Ustilago tritici* on wheat and *Ustilago nuda* on barley. The loose smut came from our own experimental fields from Vilmorin 27 winter wheat and Vindicat winter barley, respectively. For each treatment about 12 heads were inoculated. The seed of these was sown, head by head, 2½ cm. apart in the row. Although the harvesting, threshing, and sowing of each head separately takes more time, it is of great advantage that a check on the inoculation and on the possible errors made, remains possible. Such errors may occur, even when the experiments are carried out most accurately. With barley, among the 132 inoculated heads of 11 treatments, no irregularity occurred; with wheat, however, 5 among the 177 inoculated heads of 15 treatments showed some deviation. Of these 5 heads there were 4 that in subsequent culture (116 plants) showed no loose smut at all, 1 that in subsequent culture of 38 plants showed but 1 smutted plant, while the other heads of the same treatments had a high percentage of loose smut. Evidently either wrong heads were marked or wrong heads were harvested.[3] If the heads of one treatment had not been harvested and sown separately, but together, such errors would not have come to light and the percentage of loose smut would have been considerably lower than when such mistakes are eliminated. For the treatments in which the errors occurred, the percentage of loose smut would have been 78.6, 58.4 and 77.3, had the errors not been considered. After elimination of the deviating heads these percentages were 86.0, 73.4, and 80.9, respectively, (cf. Table 2, notes a, b, and c).

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[2] The mixture used consists of 50 per cent of wax and 50 per cent of vaseline. In hot weather the percentage of wax may be a little higher.

The sowing took place at the end of October in a greenhouse with loose frames in good, damp garden soil heavily manured with stable manure. The seed emergence was excellent. In the beginning of December the glass was removed and, because of a relatively warm winter, it was not necessary to put the glass on again. The crop tillered strongly and began to lodge in spots before heading. The plants that lodged early headed only in part; so that later on it was not possible to examine and determine whether all plants were attacked by loose smut. A number of plants, therefore, had to be ruled out, which, however, owing to the large number of remaining plants, did not affect the results very much.

The controls could not be sown under the same conditions, but had to be sown in the field, together with the seed of an experiment with different varieties.

Before sowing, the seeds were counted. After emergence, and in June, the plants were counted to determine the loss of plants at emergence and during winter.

The percentages of loose smut were calculated for subsequent culture of each head separately and for the plants of the 12 heads of each treatment together. The percentages as shown by the subsequent cultures of the separate heads have been omitted here for lack of space. They are, however, indicated by dots in figures 2 and 4. Thus, some idea may be gained in regard to the difference in attack of the individual heads of each treatment. But it should be borne in mind that the number of plants deriving from one head varies strongly, so that one dot has more value than the other. This marked variation in number of plants deriving from one head is not a result of the number of grains harvested, but of the fact that, owing to early lodging of several plants, their infection could not be established.

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[3] The occurrence of loose smut in subsequent culture of a non-inoculated wheat head can be explained from the presence of wheat with loose smut in the neighborhood of the experimental plot. Also, in the control, some loose smut occurred.

In the percentages of loose smut calculated according to the total number of plants of each treatment (mostly deriving from 12 heads), the mean error also is given.

ENTIRELY OR PARTLY DISEASED PLANTS

In June, for the subsequent culture of each head of each treatment, the number of diseased and healthy plants was separately noted. Most plants were either entirely healthy or entirely diseased. A part, however, showed either a number of diseased and healthy heads in the same plant or one or a few partly diseased heads in the same plant. These plants were called partly diseased. A distinction was made between plants that showed as many diseased heads as healthy ones or more diseased ones (degree of attack B) and plants that showed more healthy than diseased heads (degree of attack C). To the entirely diseased plants (degree of attack A) were counted also those that among wholly smutted heads showed at most one head that was less than half smut-free. As healthy plants only those were counted that showed no trace of smut.

If, in the determination of the degree of attack there were partly smutted heads these were counted as half-diseased, half-healthy heads.

The various degrees of attack may be indicated as follows:

A. Entirely diseased.	All heads diseased or at most one head less than half healthy.
B. Partly diseased.	Half of the number of heads or more, diseased (one partly diseased head = a half-diseased, half-healthy head).
C. Partly diseased	Less than half of the number of heads diseased.
D. Healthy.	Entirely healthy.

In the barley experiments no distinction has been made between the degrees B and C, because nearly all the plants belonged to degree B. Most of these partly diseased plants showed some partly healthy heads, while the number of plants with one or more healthy heads, together with some entirely diseased heads, was comparatively small.

For wheat it was just the reverse. Here, in most plants of degrees B and C, smutted and smut-free heads occurred in one and the same plant, while partly diseased heads were much rarer. For wheat, a distinction was made between the degrees B and C.

Of the 2733 diseased plants of wheat (15 treatments) 2633, or 96.3 per cent, were entirely diseased, 94, or 3.7 per cent, showed the degree of attack B and 6, or 0.2 per cent, the degree C.

For barley these numbers are:

2290 diseased plants (11 treatments).

Attack A: 2203, or 96.2 per cent; attack B and C: 87, or 3.8 per cent.

Attack C was recorded as less than 0.2 per cent.

In calculating the percentage of loose smut the wholly and partly smutted plants were counted together. Since the percentage of partly diseased plants probably is not affected by the period of inoculation or the concentration of the spore suspension, the data for each separate treatment are omitted.

LOSS OF PLANTS AT EMERGENCE AND DURING WINTER

Before discussing the experiments proper, something must be said here about plant mortality at emergence and during winter. According to Thren (4) losses of 20 to 50 per cent or more are mentioned in the literature. In contrast with this only a very small loss was found under the above conditions. A comparison with plants sown in the field under normal conditions could not be made, because the plants in the field—inoculated ones of an experiment with different varieties, as well as controls of the experiments described in this paper—suffered greatly from wire-worm damage. It could not, therefore, be established whether the slight loss of plants should be ascribed to the specially favorable greenhouse conditions or to the method of inoculation.

In figure 2, which refers to the experiments with wheat, the number of plants at emergence and the plants in mid-June, are indicated for each treatment separately. The figure shows that neither the concentration nor the period of inoculation has any effect on the number of surviving plants. From table 1 it appears that the loss averages only 9.1 per cent.

TABLE 1.—*Comparison of the number of sown grains, emerged plants, and the number of plants in June of wheat and barley inoculated with loose smut*

Variety	Number of treatments	Number of heads harvested	Number of grains sown end of Oct., 1937	Number of plants emerged in % of the number of seeds	Number of plants mid-June in % of the number of seeds
Wheat, Vilmorin 27	15	177	7533	95.8	90.9
Barley, Vogels Agaer	7	84	4846	91–98	83–96
Barley, Vindicat 14	4	48	2585	89–98	82–92

For barley things are slightly different (Fig. 4). In the experiment on the period of inoculation it appears that a high percentage of loose smut is correlated with a slightly larger loss of plants. Further, it is obvious that a higher concentration causes a larger loss than a lower one, whereas the percentage of loose smut remains the same. In both cases the total maximum loss does not yet amount to 20 per cent (Table 1), so that this is twice as much, it is true, as for wheat, but not so large as the losses mentioned in the literature.

EXPERIMENTS WITH LOOSE SMUT OF WHEAT

The Optimum Period of Inoculation. In a plot of winter wheat, variety Vilmorin 27, many heads in the same stage of development were marked at the beginning of anthesis. Of these heads 12 were inoculated every 1–3 days. The first inoculation took place on June 9, 1937, when the wheat was in mid-anthesis. On June 12 (3rd inoculation) the flowering period was about over. The 7th and last inoculation took place on June 21. From June 9–13 the weather was hot to very hot, dry and bright. Thereafter, cloudiness increased with now and then rain and with much lower temperature. In table 2 and figure 2 the results of the experiment are mentioned. The effect of the inoculation depends much on the time the spores are applied. The most favorable time is during mid-anthesis. With the 3rd inoculation the percentage of loose smut has already decreased by more than one half. It is not certain whether the inoculations on June 19 and 21 produced any smut. Such as was observed, viz., 2.8 and 1.6 per cent, may also have been the result of infection coming from a neighboring plot, just as this must have been the case in the control.

A consideration of the number of partly diseased plants expressed in percentage of the total number of diseased plants shows that there is hardly any relation between the percentage and the greater or lesser chance of a successful infection. The figures found in the case of the inoculation on June 19 and 21, viz., 11.1 and 28.6 per cent, seem to make an exception, but it should be borne in mind that these percentages are calculated from a very

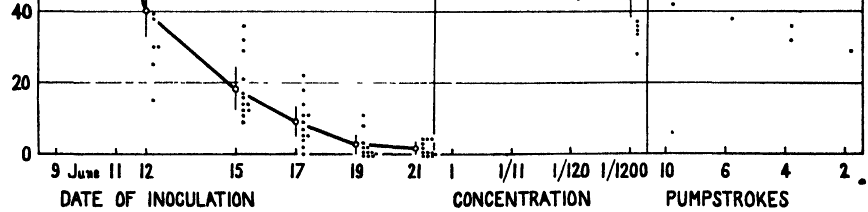

FIG. 2. Results of the inoculation experiments with loose smut on Vilmorin 27 winter wheat. Squares, number of plants at emergence in per cent of the number of grains. Crosses, number of plants in June in per cent of the number of grains. Circles, percentage of smutted plants; the vertical line represents three times the mean error. The dots (●) indicate the percentage of loose smut in the after culture of each head separately. The percentage of smut of each treatment was calculated from the total number of plants and is not the average of the percentage of smut of the separate heads.

small number of diseased plants, viz., 9 and 7, respectively. The obvious surmise that with a late inoculation the number of partly diseased plants increases is, therefore, not confirmed.

The Concentration of the Spore Suspension. The highest concentration used in my experiments was 1 g. spores per l. of water, a quantity about equal to or slightly less than that taken for the ordinary inoculations. The suspension was dark brownish-black and completely opaque. This concentration was called 1, and from it the dilution 1/11 was made with the aid of a pipette after shaking well. From this dilution a further dilution of 1/120 was obtained in the same way, and from this again a dilution of 1/1200. In view of the instability of the suspension (the spores fairly soon sank to the bottom) the concentrations were as far as possible checked with the counting chamber according to Thoma. As appears from table 3, the agreement is reasonably good. Figure 3 shows the different suspensions made later on, viz., the concentrations 1, 1/10, 1/100 and 1/1000. The inoculations were performed on June 10 in the same plot of wheat as mentioned above. The results of the experiment with the different concentrations (Table 2 and Fig. 2) show that a concentration of 1/11 still gives about a maximum effect. In lower concentrations the effect decreases. Yet, with a concentration of 1/1200 (i.e. about 10 spores per cu. mm.) the percentage of loose smut still amounts to 45.

TABLE 2.—*Results of inoculation experiments with loose smut of wheat. Variety Vilmorin 27*

Treatment	Total number of plants	Number of diseased plants	Percentage smutted plants
Date of inoculation			
9 June '37	357[a]	307	86.0 ± 1.8
11 " "	391[b]	287	73.4 ± 2.2
12 " "	415	167	40.2 ± 2.4
15 " "	367	67	18.3 ± 2.0
17 " "	444	40	9.0 ± 1.4
19 " "	324	9	2.8 ± 0.9
21 " "	429	7	1.6 ± 0.6
Concentration of spores			
1	303	252	83.2 ± 2.2
1/11	346[c]	280	80.9 ± 2.1
1/120	441	260	58.9 ± 2.3
1/1200	484	220	45.4 ± 2.3
Number of pumpstrokes			
10	343	286	83.4 ± 2.0
6	273	186	68.1 ± 2.8
4	270	177	65.6 ± 2.9
2	341	188	55.1 ± 2.7
Control, 6 rows of 3 m., one smutted plant.			

[a] 36 plants, deriving from one head, showed for some uncheckable reason no smut at all and were therefore eliminated.

[b] In 102 plants, deriving from 3 heads, there was only one smutted plant. Plants eliminated.

[c] 16 plants, deriving from one head, showed no smut and were therefore eliminated.

The Number of Pump Strokes. Whereas normally 10 pump strokes were

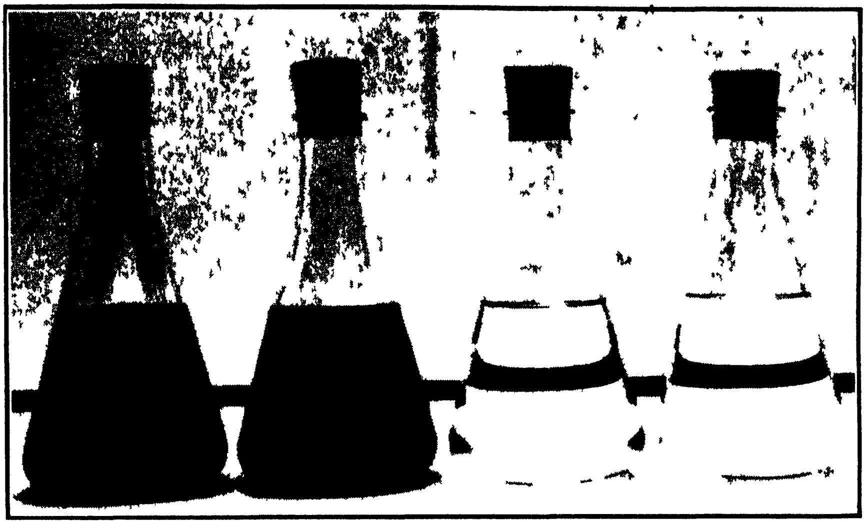

FIG. 3. Spore suspension of *Ustilago tritici* in water. From left to right the concentrations 1, 1/10, 1/100 and 1/1000. The concentration 1 contains 1 gr. spores per liter water.

given, with this experiment the number varied from 2 to 10. The date of inoculation was June 10, same plot as above. Table 2 and figure 2 give the results. With 2 pump strokes the number of smutted plants is already as high as 55 per cent, with increase of the number of pump strokes to 10 the number of smutted plants increases to 83 per cent. It is not certain whether with this number the optimum is already reached Moore (1) indicates that 4–6 strokes are sufficient, but he was working under different conditions. In his experiment the normal pressure after each pump stroke was restored, which was not so in our case. It is not impossible that, owing to the great changes of pressure, Moore reached the optimum effect sooner.

EXPERIMENTS WITH LOOSE SMUT OF BARLEY

The Optimum Period of Inoculation. This experiment was planned in the same way as for wheat. The first inoculation took place in a plot of Vogels Agaer winter barley on May 27, whilst the immature heads were still partly hidden in the boot. With the second inoculation (May 29), 10 of the 12 heads were completely free. The flowering was not recorded, but probably by May 31 it was largely over. Up to May 31 the weather was warm, dry, and sunny. On May 31 it was still warm but cloudy. An hour after inoculation there was a fairly heavy shower of rain. From June 1–4 the weather was dry and cool, followed by alternate hot to very hot temperature with occasional showers of rain, not, however, immediately after the inoculation.

In table 4 and figure 4 the results are summarized. It will be noticed that the moment of inoculation is of great importance. In 2 days the percentage of loose smut decreased from its maximum (84 per cent) down to 18

TABLE 3.—*The concentration of the spore suspensions calculated from countings with the counting chamber of Thoma. Loose smut of wheat and loose smut of barley*

Concentration according to the dilution	Colour of the suspension	Number of samples × counted volume in cu. mm.	Number of spores		Ratio calculated from column 5
			counted	per cu. mm.	
		Wheat			
1	Opaque brownish-black	5 × 0.005	478	19120	1
1/11	Greyish-brown	5 × 0.05	404	1616	1/11.8
1/120	Fairly transparent	11 × 0.1	137	125	1/153
1/1200	Completely transparent	....	....	. ...	....
		Barley			
1	Opaque brownish-black	2 × 0.02	682	17050	1
1/10	Greyish-brown	13 × 0.1	1665	1280	1/13.3
1/100	Fairly transparent	10 × 0.1	96	96	1/178
1/1000	Completely transparent	.. ..	....	....	. . . .

per cent. For barley, therefore, even more than for wheat, the inoculum should have been applied at the right moment. The few smutted plants after the inoculation of May 31 was, however, partly attributable to the weather, as pointed out above.

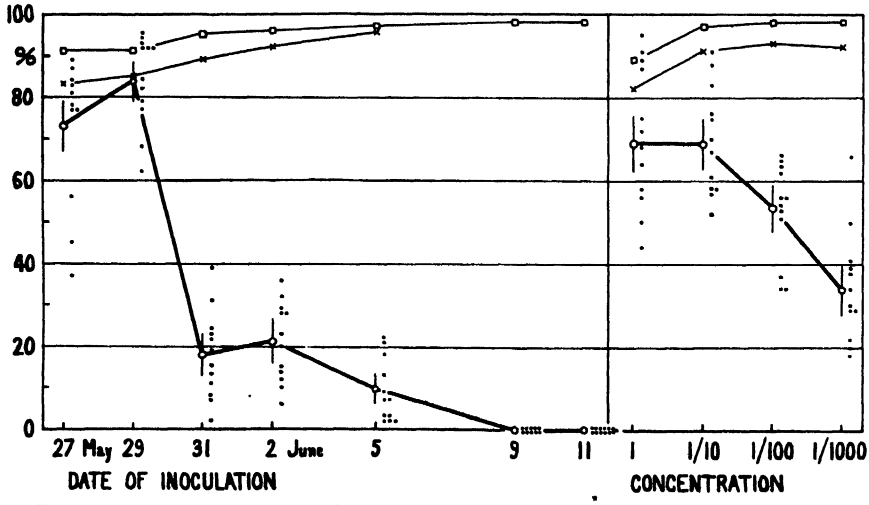

FIG. 4. Results of the inoculation experiments with loose smut on winter barley. Squares, number of plants at emergence in per cent of the number of grains. Crosses, number of plants in June in per cent of the number of grains. Circles, percentage of smutted plants; the vertical line represents three times the mean error. The dots (●) indicate the percentage of loose smut in the afterculture of each head separately. The percentage of smut of each treatment was calculated from the total number of plants and is not the average of the percentage of smut of the separate heads.

The Concentration of the Spore Suspension. In the case of loose smut of barley, too, the highest concentration used was 1 g. spores per l. of water. The dilutions made were likewise checked by counting the spores (Table 3).

TABLE 4.—*Results of inoculation experiments with loose smut of barley*

Treatment		Total number of plants	Number of diseased plants	Percentage smutted plants
	Date of inoculation			
Variety Vogels Agaer	27 May 1937	476	348	73.1 ± 2.0
	29 " "	562	471	83.8 ± 1.6
	31 " "	523	95	18.1 ± 1.7
	2 June 1937	538	114	21.2 ± 1.8
	5 " "	556	52	9.4 ± 1.2
	9 " "	not counted	0	0
	11 " "	not counted	0	0
	Concentration of spores			
Variety Vindicat 14	1	427	295	69.1 ± 2.2
	1/10	529	364	68.8 ± 2.0
	1/100	680	364	53.5 ± 1.9
	1/1000	553	187	33.8 ± 2.0

Control Vogels Agaer, 8 rows of 3 m., and Vindicat 14, 6 rows of 3 m.; no smut.

For this experiment a plot of winter barley, variety Vindicat 14, was inoculated on May 28. The results deviate slightly from those obtained for loose smut of wheat. With concentration 1/10 the effect is still optimum, but it decreases gradually with decreasing concentrations. It may further be pointed out that with concentration 1 the loss of plants during winter is larger than with concentration 1/10, so that for barley, and especially for winter barley, it is of importance not to inoculate with too high a concentration. This is in agreement with the investigations of Thren (4), in which, however, the differences between the application of different concentrations were found to be much larger.

SUMMARY

For the inoculation of wheat and barley with loose smut, Moore's method, with some modifications, was applied. A considerable improvement was obtained by putting into the inoculating chamber 4 heads instead of one. When 2 persons are working together the speed can thus be increased up to 80 heads per hour for wheat and 50 for barley, including selecting and marking of heads. In this respect Moore's method surpasses the Halle method. The apparatus and experimentation are adequately described.

In contrast to the experiences of others, under our experimental conditions the loss of plants at emergence and during winter was only slight. For wheat the loss at emergence and during winter amounted to an average of 10 per cent, regardless of the period of inoculation and of concentration of spore suspension. In barley there was some relation between the loss of plants and the period of inoculation or the concentration used. However, the total maximum loss did not yet amount to 20 per cent.

In all experiments partly diseased plants, degrees B and C, were observed. For wheat the number was 3.9 per cent, for barley 3.8 per cent. Of these partly diseased plants only a small part was less than half diseased

(for wheat 0.2 per cent of degree C to 3.7 per cent of degree B; for barley the percentage of degree C was even smaller). The way in which plants were partly diseased was different for wheat and barley. In Vilmorin 27 winter wheat in most cases healthy and entirely diseased heads occurred in the same plant, in winter barley partly diseased heads were more prevalent. There probably is no relation between the occurrence of partly diseased plants and the period of inoculation or the concentration of the spore suspension.

The optimum period for inoculation lasts only a few days and during anthesis. The spore inoculum has a maximum effect both for wheat and for barley in a concentration of 1 g. and 0.1 g. spores per l. of water, respectively. With concentrations of about 0.001 g. spores per l. water, or about 10 spores per c.mm., a fairly good infection still takes place.

With an increasing number of pump strokes from 2 to 10, the number of smutted plants increases (experiments only with wheat). Whether the maximum is already reached with 10 pump strokes is not certain, but for practical reasons a greater number is not recommended.

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STUDIES ON "DAMPING OFF" OF CULTIVATED MUSHROOMS AND ITS ASSOCIATION WITH FUSARIUM SPECIES II

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INTRODUCTION

In an earlier paper (6) dealing with this subject, it was shown by inoculation experiments that mushrooms failed to develop normally if casing soil, infected with species of *Fusarium*, was used on the mushroom beds. *F. martii* and *F. oxysporum* were studied in detail, but further cases were cited where other species of *Fusarium* in the casing soil showed typical "damping off" symptoms.

Reference to the literature dealing with the *Fusaria* and their relations with other plants indicate that the majority of workers in this field consider pathological conditions to be attributable primarily to the excretion of toxins (1, 2, 3, 4). The investigation here reported was carried out in an attempt to show the relationships between *Agaricus* and the various *Fusarium* species in cases of "damping off."

Other factors that seemed to favor its development were investigated in an endeavor to determine effective control measures.

TECHNIQUE

Two facts had first to be demonstrated.

1. That antagonism existed between the various species of *Fusarium* (isolated from infected casing soil) and *Agaricus*.
2. To show if possible that the degree of antagonism varied with the species of *Fusarium* present.

The ideal apparatus for the purpose was found to be the special spawn jar patented by the Chester County (Pennsylvania) Mushroom Laboratories, who very kindly gave permission for its use. The jars were filled to a depth of 6–7 inches with prepared compost, sterilised, and inoculated with a brown strain of *Agaricus campestris*. During the period in which the spawn was running through this compost the casing soil was prepared as follows:

A quantity of corn-meal-sand medium was seeded with a spore suspension of the respective *Fusarium* species and incubated.

A good quality clay loam was sifted through a ½ in. mesh screen, slaked lime was added to bring the reaction to pH 7.2, and the mixture was sterilized in the autoclave at a pressure of 17 pounds for ½ hour. When cold, 1 part of the inoculated corn-meal-sand medium was thoroughly mixed under aseptic conditions with 15 parts of the sterilized soil, and the whole was used to case the spawn in the jars.

For the purpose of the experiment, instead of the usual 1 in. of soil, as used by the commercial mushroom growers, 3 in. of casing soil was applied in order to give a sufficiently deep area in which the growth of the two mycelia could be observed. The jars were cased with soil inoculated with *Fusarium culmorum*, *F. martii*, *F. oxysporum*, *F. flocciferum*, and *F. dianthi* and were kept at 55–60° F. In effect each jar showed a section of a mushroom bed, 6 in. deep cased with 3 in. of casing soil. A control jar was set up, cased with similar soil but noninoculated.

In every case the *Fusarium* mycelium spread rapidly through the soil until it met the upward growing mushroom mycelium. This latter then ceased growth and became quiescent for some weeks or senescent in the more extreme cases.

Moreover, a definite line of demarcation became visible, after a few weeks, at the junction of the spawn and the casing soil, the mycelium in this region tending to rot and form a deep brownish layer. The rest of the spawn underlying this area assumed a reddish tint (instead of the healthy bluish white appearance) this color being usually associated with adverse conditions in the bed, *e.g.*, high alkalinity (pH 7.9). In the control jar the spawn grew normally into the soil and produced mushrooms. The results are shown in figure 3, A–F.

The preceding experiment showed that where a species of *Fusarium* and *Agaricus* are in simultaneous competition, the *Fusarium* grows rapidly and

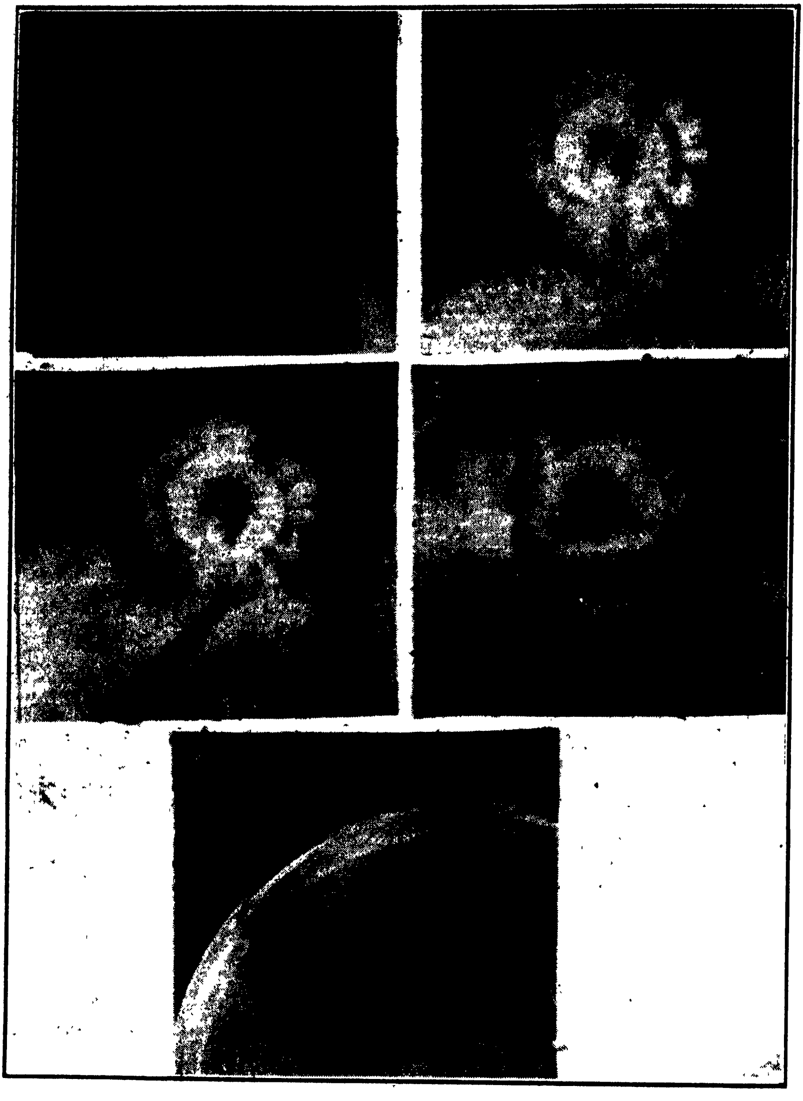

FIG. 1. Antagonism between *Agaricus* and *Fusarium culmorum*. A. Antagonism caused an indentation in the margin of the *Fusarium* culture (lower colony). Dec. 11, 1937. B. *Fusarium* hyphae advance and begin to encircle *Agaricus* culture. Dec. 15. C. *Fusarium* hyphae become recurved (see arrow) on meeting with *Agaricus* hyphae. Dec. 20. D. Mycelium of *Fusarium* massed on either side of *Agaricus* culture to a depth of 5 mm. Dec. 31. E. Under surface of Petri dish showing the periphery of the *Agaricus* colony delineated by scarlet pigment. A, $\times \frac{2}{3}$; B–E, ×1.

checks the mushroom spawn. It was thought possible that if *Agaricus* was first well established before the *Fusarium* infection became apparent, then possibly the antagonism created by the mushroom spawn over a period would effectively check *Fusarium* growth, *i.e.*, that antagonism was reciprocal.

It is well known that a big difference in the growth rate exists between the *Fusarium* mycelium on one hand and that of *Agaricus* on the other. Growth curves, obtained by measuring the increase in diameter of cultures of *Agaricus* (brown variety) and those of *F. oxysporum* and *F. martii* every 24 hours to ensure a correct growth curve (5), showed that the ratio of the growth rate of *Fusarium oxysporum* and *Agaricus* is approximately 3 : 1 and of *F. martii* and *Agaricus* 4 : 1. All other species of *Fusarium* similarly tested gave approximately similar results. It is to be expected therefore that under the conditions prevailing in a mushroom house the growth rate of the *Fusarium* contaminants will greatly exceed that of *Agaricus* when infected casing soil is placed on the beds.

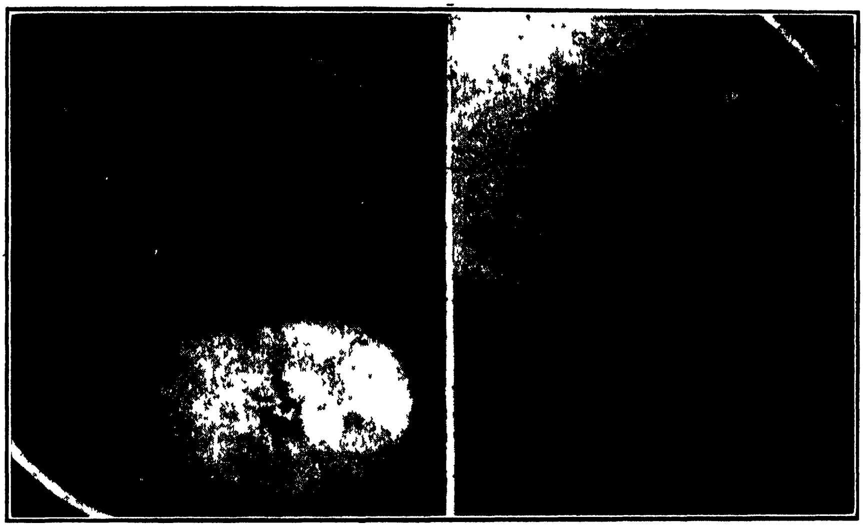

FIG. 2. Antagonism between *Fusarium oxysporum* and *F. martii* and *Agaricus*. × approximately 1. A. The two cultures showing the hyphae in close contact. Note the recurving of the *F. oxysporum* hyphae (upper colony) to form a distinct ridge. B. *F. martii* and *Agaricus* showing the indentation of the *Fusarium* colony (upper culture).

It was shown by preliminary experiments that if *Agaricus* and a *Fusarium* species were inoculated into opposite sides of a 5 per cent malt-agar plate at the same time, little or no antagonism could be perceived between the 2 fungi, as the *Fusarium* rapidly outgrew the *Agaricus* and overcame what little resistance was offered. Bearing in mind the discrepancy in growth rates it was found that if the *Agaricus* was allowed to grow alone for some time until sufficient growth had taken place to allow an accumulation of metabolic waste products a different result was obtained.

Plates of 5 per cent malt agar were poured using fairly large Petri dishes (10 cm. diam.) Each dish was inoculated at one side with mushroom spawn which was allowed to grow at room temperatures (60° F.) until it had formed a colony approximately 2 cm. in diameter. The *Fusarium* species used was then inoculated at the opposite side of the dish, so that the two mycelia spread towards the centre. For this experiment *F. martii*, *F. culmorum* and *F. oxysporum* were used in turn.

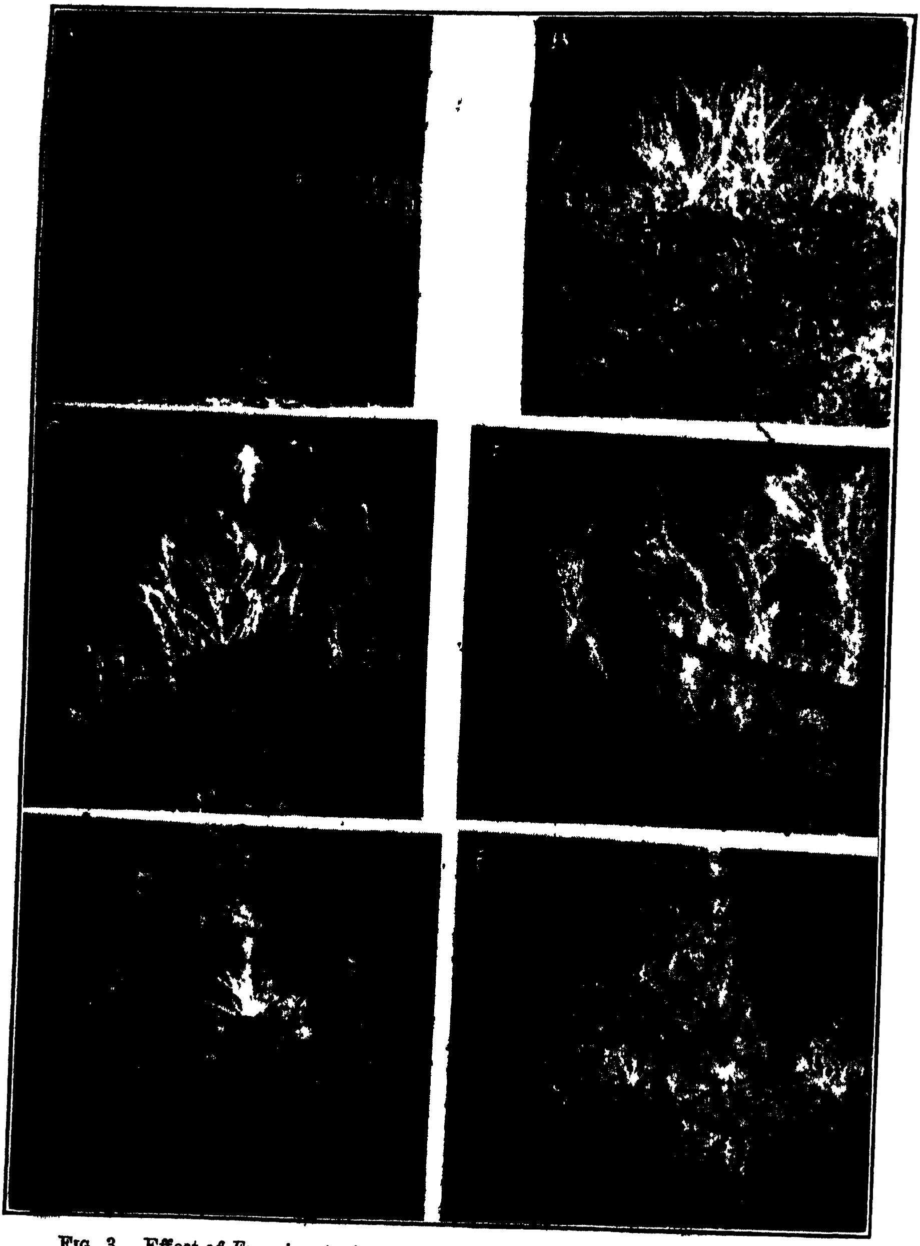

FIG. 3. Effect of *Fusarium* toxins in casing soil on growth of *Agarious*. Approx. × 1.
A. *F. dianthi*. Strand formation in *Agaricus* mycelium. B. Same as A but 1 month
later. Little further upward growth but note the heavy thread formation. C. *F.*
culmorum. Mycelium forms a typical heavy-stranded frond like formation. C. *F. oxy-*
sporum. Thick white strands, which penetrate only a short distance into the soil. Note how
mycelium avoids heavily infected patches in the soil (see arrow), which show the charac- D. *F.*
teristic red pigmentation (*cf.* Fig. 1, E). E. *F. flocciferum*. Spawn growth very poor
and stunted. F. *F. martii*. Spawn development more uniform in general and the soil is
nearly filled with mycelium. The growth is very little different from a normal *Agaricus*
growth in noninfected soil.

Fusarium culmorum gave a most striking result and one that was, unfortunately, very difficult to illustrate to perfection in a photograph. The *Fusarium* mycelium, on reaching a distance of 3 mm. from the *Agaricus* mycelium, not only grew no further on the medium but the aerial and subaerial hyphae actually became recurved.

The series of photographs (Fig. 1, A–E) show quite clearly the way in which antagonism developed. The final photograph shows the *Fusarium* mycelium massed around the *Agaricus* colony and forming a bank of hyphae 5 mm. high. Looking at the under surface of the Petri dish the area of antagonism was delineated by the deposition of a brilliant scarlet pigment by the *Fusarium,* and the *Agaricus* colony was thus seen through the base of the Petri dish outlined in scarlet (Fig. 1, E).

Finally, the *Fusarium* mycelium was found to be growing on top of the *Agaricus* colony, but there was still a distinct break left between the *F. culmorum* hyphae surrounding the colony and those hyphae growing *on* the *Agaricus* colony. This showed clearly that the invasion by the *Fusarium* mycelium had taken place by the germination of spores that had been shaken onto the surface of the *Agaricus* colony, and also that the immediate vicinity of the colony was still antagonistic to growth of the *Fusarium* hyphae immediately surrounding it.

In the case of *Fusarium oxysporum* it was found that the growth of the mycelium came to a standstill when it was within 2 mm. of the *Agaricus* mycelium and the antagonism was so pronounced that, when the *Fusarium* mycelium had eventually filled up the rest of the Petri dish, the colony of mushroom spawn was still isolated and half encircled by the *Fusarium* mycelium (Fig. 2, A).

Fusarium martii was used in the same way, and a similar type of result was obtained, though the demarcation was less distinct (Fig. 2, B).

The growth curves of *Fusarium culmorum* and *Agaricus* grown on the same dish as described, were plotted and the graph shows distinctly (A, B) the cessation in growth when the mycelia were in close contact, in which case the distance was 5 mm. (Fig. 4, A).

Similar results were obtained with other *Fusarium* species and at different temperatures the only difference being the degree of intensity of antagonism. Figure 4, A, shows the type of result obtained at 18° C., while figs. 4, B, and 4, C, show results at 32° C. In both of these graphs the characteristic flattening of the *Fusarium* growth curve will be seen at A.

EFFECT OF FUSARIUM WASTE PRODUCTS ON GROWING MUSHROOMS

The preceding experiments showed that both *Agaricus* and *Fusarium* are mutually antagonistic and the next step was to demonstrate that the antagonism in the casing soil is due to substances excreted by the *Fusarium* which are toxic to *Agaricus.*

To investigate this effect dishes were set up in exactly the same manner as described in a previous paper (6).

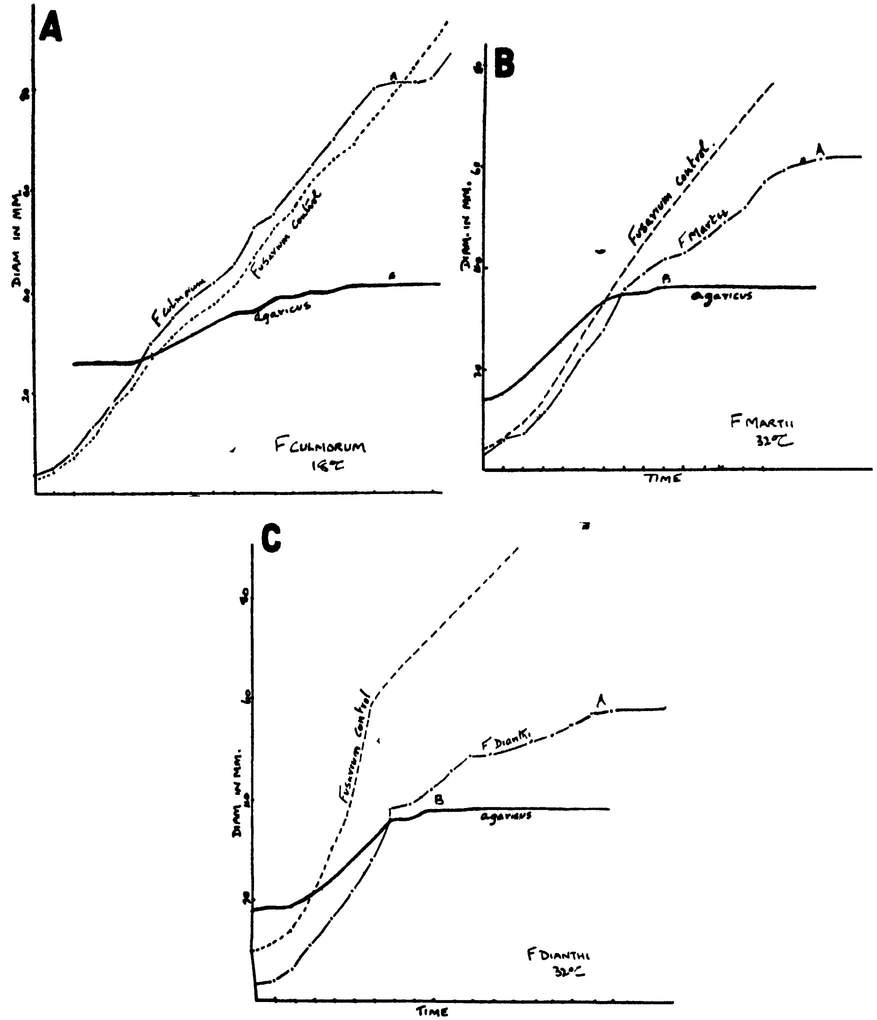

Fig. 4. Growth curves of *Fusarium* spp. and *Agaricus*. A. Cessation of growth when mycelia of *F. culmorum* and *Agaricus* were in close contact; cultured at a temperature of 18° C. B. Same for *F. martii* and *Agaricus* at 32° C. C. Same for *F. dianthi* and *Agaricus* at 32° C.

At the same time the culture dishes were set up Erlenmeyer flasks were prepared containing Richard's solution. As *Fusarium culmorum* showed such marked antagonism to mushroom spawn this species was used for the experiment and the flasks were inoculated with it after sterilization.

The fungus cultures were kept under the same conditions as the culture dishes and were allowed to grow until staling took place. One of the flasks was inoculated with a culture of *Chromobacterium prodigiosum* and the contents were filtered through a Berkefeld filter candle into a flask, the whole of the filtering apparatus having been previously sterilized. Loopfuls of this filtrate were plated onto nutrient agar slopes and incubated at 30° C. for

24 hr. On inspection the slopes were found to be sterile, showing that the filtrate was sterile.

Two dishes were sprayed with the *Fusarium* extract each time watering of the casing soil was necessary. Two dishes were sprayed with sterile distilled water and 2 others with sterile Richard's solution. The times at which the *Fusarium* toxins were added to the soil were varied in order to demonstrate whether a time factor determined the extent of "damping off" induced by the excreted *Fusarium* toxins.

In the first experiment the addition of the *Fusarium culmorum* extract was delayed a month, or until the spawn was entering the casing soil (Table 1).

TABLE 1.—*Effect of Fusarium culmorum extract on mushroom production; cultures sprayed one month after spawning. Cultures B1 and B4 are controls watered with sterile water; B5 and B6 are controls watered with sterile Richard's solution*

Date	Weight of mushrooms in grams					
	B1	B2	B3	B4	B5	B6
May 24	65.9	31.1			25.2	
May 25		[a]	69.9[a]	31.7		20.5
May 29				20.0	12.1	19.4
May 30					10.4	
June 1		85.2				20.0
June 2	30.1	(A)[b]			20.6	12.1
June 4			50.5	34.8		
June 7	2.0	0.5	2.3	0.5(A)	3.0	1.9
June 10	40.5				30.8	
June 11				36.5		
June 12			18.5			
June 14		34.1			19.4	30.6
June 21			59.7		10.6	
June 22		14.7		38.4		15.5
June 23						24.2
June 24						16.6
June 25	19.7	10.0			26.7	
Totals	158.2	175.6	200.9	161.9	158 8	160.8

[a] Sprayed with sterile *F. culmorum* extract after 1 month.

[b] (A) = Characteristic dark nigger brown; buttons rubbery in texture. All recased with sterilised soil June 17.

These results were checked by repeat experiments and it was found that there was always a comparable discrepancy between the inoculated dishes and the controls.

As a control experiment to compare results obtained with the living fungus with those obtained by using the sterile extract an experiment was set up in which the soil was sprayed with *Fusarium culmorum* spore suspension from the commencement.

The total results were: Culture B1/97, 154.7 g.; culture B2/97, 116.9 g.; culture B3/97, 163.9 g. B1/97 and B2/97 were the inoculated dishes and *both showed marked "damping off" symptoms,* B3/97 was the control dish.

From these experiments it would seem that if addition of the *Fusarium* toxins was delayed until spawn growth and, therefore, *Agaricus* excretory products were already in the soil, the toxins had a stimulating rather than an inhibitory effect on mushroom production. "Damping off" symptoms were *present, however, and it would seem that the products responsible for this are capable of acting as physiological stimulants when present in small quantities or when the Agaricus resistance is high, and as poisons when present in large excess or when Agaricus resistance is low.*

To show this a further experiment was carried out in which the casing soil was watered with the sterile *Fusarium* extract as soon as the culture dishes were set up. The experiment was repeated several times and typical results are presented in table 2.

TABLE 2.—*Mushroom production in cultures sprayed with Fusarium culmorum extract compared with production on control dishes. Cultures B1 and B4, sprayed with F. culmorum extract; B2 and B3, controls, sprayed with sterile water; B5 and B6, controls, sprayed with Richard's solution. Experiment set up on June 15, 1937*

Date	Weight of mushrooms in grams					
	B1	B2	B3	B4	B5	B6
			All cultures pipping up			
June 29	........[a]	....	....	..[a]	.	
July 7	34.0	31.3	.	48.3	33.0	
July 15	....	....	96.6	......	..	70.6
July 16	44.0	65.0	....	18.4	40.0	26.3
July 17	36.7	....	....	45.7	.	
July 26	....	24.8	. ..	....	26.2	24.9
July 28	......	....	24.9	....	22.2	....
Totals	114.7	121.1	121.5	112.4	121.4	121.8

[a] Mycelium not so strongly marked in the soil in B1 and B4 (*cf.* Fig. 3).

It is thus indicated that the presence of *Fusarium culmorum* toxins in the casing soil causes a similar diminution in yield and the same characteristic features in the mushroom as does the living fungus and the effect of the toxins is modified if their addition is delayed until the beginning of production.

EFFECT OF OXYGEN CONTENT ON AGARICUS AND FUSARIUM SPECIES

The great majority of mushroom growers in England use nonsterilized casing soil on their mushroom beds. The casing soil is applied about 14 days after spawning, and cropping commences under normal conditions from 3–4 weeks after casing. Where a normal crop results one may assume that the growing conditions are ideal and that the huge excess of mushroom mycelium then overgrows any contaminants in the casing soil.

In cases where "damping off" takes place at the beginning of the crop it would seem that some factor is present that either accelerates the growth of *Fusarium* or inhibits that of *Agaricus*. In many instances it was noticed that the following factors were consistently present.

a. Heavy clay casing soil, finely screened prior to application. Absence of lumps causes the soil particles to cohere on watering; the mushroom-house temperature dries the surface of the casing soil to a hard crust, which forms a very efficient seal between the air in the house and the spawn in the compost beneath.

b. A very common mistake is lack of ventilation during the early stages of mushroom cultivation, caused either by insufficient air vents or by stagnant air due to inefficient air circulation.

Experiments were undertaken to determine the effect of the presence or absence of oxygen on *Fusarium* species and *Agaricus* mycelia. For test purposes *F. oxysporum* was used. A preliminary experiment was carried out by inoculating two slopes of Dox agar with *F. oxysporum*, one tube being incubated under anaerobic conditions. To indicate this effect more fully 2 large Petri dishes of 5 per cent malt agar were inoculated with the *Fusarium* species to be studied. After incubation at 30° C. for 24 hours, one culture plate was put under anaerobic conditions, the other left as a control. During the course of 3 days the diameter of the anaerobic *Fusarium* colony increased considerably, the most noticeable characteristic being that the mycelium was extremely fine and tenuous. Growth gradually came to a standstill until aerobic conditions were induced when the growth rate became normal. *F. martii, F. oxysporum, F. culmorum, F. dianthi* and *F. flocciferum* were tested in this manner, all with similar results.

A similar experiment was set up under the same type of conditions using *Agaricus;* and in this case, because of the slowness of growth of the mycelium, the culture was allowed to develop until vigorous growth obtained, anaerobic conditions not being set up until 10 days after inoculation. After 4 days, under anaerobic conditions, the culture was aerated, but no further growth took place; examination showed that the culture was dead.

The experiments show that oxygen deficiency will check the growth but not necessarily injure the common *Fusarium* species causing "damping off." If the condition of oxygen deficiency is too prolonged the mycelium is killed outright.

THERMAL DEATH POINT AND PREVENTATIVE MEASURES

With a view to preventative measures experiments were made to discover the thermal death points of *Fusarium martii* and *F. oxysporum*—these being the two common causes of "damping off." The usual procedure was adopted and cultures were then plated out, using Dox agar, and incubated at 30° C. The thermal death point of *Fusarium martii* lies between 47–48° C. at 20 min. or 50–51° C. for 5 min. That of *F. oxysporum* is 55° C. for 20 min. or 58–59° C. for 5 min. Similar tests with other *Fusarium* species concerned in "damping off" showed that if the soil used for casing was subjected to steam heat, the temperature being maintained at 60–70° C. (140–150° F.) for 20–30 min., this treatment would render the soil free from *Fusarium* contamination.

The author has found that sterilization of casing soil has usually eliminated "damping off" from nurseries where the problem had developed to such serious proportion that the abandoning of mushroom cultivation entirely was seriously considered.[1] Control of the trouble is very difficult because of

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[1] Since writing the above the writer has had some very striking evidence to hand to show the prevalence of *Fusarium* "damping off" and its control by sterilization. Five different mushroom growers in the Worthing district were concerned and the source of the whole of the casing soil in question came from the same site at Brighton, Sussex.

A filled one large house, shelf type, capacity 3,600 sq. ft. of bed space, casing with the soil in question. The whole house was a complete commercial failure and had to be the readiness with which the spawn succumbs to any measures devised for control of the *Fusarium*. By the time "damping off" has been observed it is usually too late (from an economic standpoint) to save the crop.

DISCUSSION

The problem of "damping off" of cultivated mushrooms resolves itself into a recognition of the existence of intense antagonism between the mycelia of the two fungi concerned—*Agaricus* and species of *Fusarium*. Once this fundamental idea is accepted the rest of the problem is concerned mainly with the factors that favor or retard development of one or other of the fungi, thus increasing or decreasing the amount of antagonism. Once *Fusarium* contamination has taken place it is an uneconomic proposition to go to great expense to achieve only moderate control measures and the problem is better tackled by recognizing the importance of elimination of diseased casing soil at the commencement of the crop, cleanliness throughout the whole of the cultivation routine, and maintainance of correct cultural conditions *with special emphasis on ventilation.*

If these factors are neglected, the progress of the disease is usually thus. The beds are cased with infected casing soil, the house temperature being about 70° F. Presence of a heavy clay soil and deficient ventilation in the house results in a rapid spread of the invading fungus through the whole of the casing soil and a corresponding check on mushroom spawn growth. Under normal conditions casing of the beds takes place 2 weeks after spawning and production about 5 to 6 weeks after laying down the beds. Hence, the spread of *Fusarium* in the casing soil, assuming it commences at once, goes unchecked for nearly 3 weeks before production begins. The intensity of the disease depends on the heaviness of the initial infection and the favorableness or otherwise of conditions. In standard houses, fitted with shelves, uneven ventilation usually means that some shelves show heavy loss from "damping off," while others are relatively unaffected. As stated in a previous paper (6) the effect of the trouble *is nearly always not cessation of production, but production of a commercially valueless crop.*

SUMMARY

Experiments were set up as described to show that antagonism exists between various species of *Fusarium* and *Agaricus* and that this antagonism is mutual.

Evidence is brought forward to show that when the casing soil has been infected with *Fusarium* it is antagonistic to mushroom spawn.

It is shown that, if due allowance be made for the large discrepancy in the

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thrown out. His second house, similar in type, was filled using the same soil, but previously steamed. No trace of damping off was found and in *a single month* of production he picked 1¼ lb. of perfect mushrooms per sq. ft. of bed space.

B had already cased 1,000 sq. ft. of beds in a greenhouse with the same soil prior to hearing of *A*'s failure. His other bed in the same house (1,000 sq. ft.) he cased with the same soil previously steamed. The first bed was a complete failure, the second bed is bearing perfect mushrooms. *C, D,* and *E* cased their beds, in spite of suggestions to the contrary, with the same soil nonsterilized. In each case the beds were worthless.

growth rates between *Fusarium* species and *Agaricus*, then a well-established culture of *Agaricus* is intensely antagonistic to *Fusarium* species.

By watering growing mushrooms with the extract from *Fusarium* species grown on Richard's solution it is shown that the non-living extract exerts an antagonistic effect the intensity of which depends on the time of inoculation.

The effect of lack of oxygen on both *Agaricus* and *Fusarium* is shown.

The thermal death point of the common *Fusarium* species causing "damping off" was determined, and it is suggested that soil sterilization is the best method of avoiding the disease.

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UNUSUAL DEVELOPMENT OF APPLE PERENNIAL CANKER, FOLLOWING APPLICATION OF TOXIC WOUND DRESSINGS

E. L. REEVES, M. A. YOTHERS, AND C. W. MURRAY

(Accepted for publication March 1, 1939)

The disease caused by *Gloeosporium perennans* Zeller and Childs and known as perennial canker (7) has been responsible for serious injury to apple trees and fruit in certain districts of the Pacific Northwest.[1] The fungus commonly attacks injured tissues of the host, particularly around pruning cuts, resulting in the formation of ragged lesions in the bark and outer wood of the trunk, branches, and twigs. Around the margins of these lesions the woolly apple aphid, *Eriosoma lanigerum* (Hausm.), finds suitable colonization quarters, protected by the covering of dead bark and furnished with a suitable food supply by the tender wound callus formed around the margins of the cankers.

Childs (1), Cooley (2), Güssow (3), McLarty (4), Reeves (5), Yothers (6), and others have demonstrated that the woolly aphid, feeding inside these lesions, is one of the important factors responsible for the continued growth of the fungus within individual cankers.

Two methods of controlling the woolly aphid and, therefore, the perennial canker are (1) the application of aphicidal sprays to canker lesions that

[1] The work reported upon in this paper was done at Wenatchee, Wash.

have been opened up to enable the sprays to reach the aphids and (2) the application of wound dressings after removal of dead bark to prevent aphid infestation of the callus. Biological control by means of the woolly aphid parasite *Aphelinus mali* (Hald.) is also effective.

In experiments over a period of 4 years by one of us (Yothers) involving the use of several hundred different kinds of wound dressings, only a few paints or dressings caused serious injury to the growing callus; these are of special interest and are reported in this paper.

On July 7 and 14, 1932, 6 experimental dressings were applied to several hundred cankers that had been cleaned out between June 10 and 27, on 5 large Jonathan apple trees. At the time the cankers were prepared for application of wound dressings all canker extension had definitely stopped for the season. The cankers were well distributed over the greater portion of the trees, including the trunk, scaffold limbs, branches, and twigs. The dressings, all of a semi-liquid but viscous form, were applied by means of a small paint brush, and worked carefully into the cankered area only, caution being taken to avoid applying the mixtures to the healthy bark.

The 6 experimental dressings employed in this particular series of tests differed principally in that 3 copper arsenites (stearo, palmito, and lauro) were incorporated separately in 3 of the dressings in an attempt to test their possible fungicidal qualities. Beeswax was a common ingredient in all the dressings to obtain the desired consistency. Peanut oil was used as an ingredient of 3 dressings, while tung, perilla, and soybean oils were utilized separately in the other three. Beta naphthylamine, nicotine sulphate, and anabasine sulphate were employed singly as possible aphid repellents and aphicides in the 3 dressings not containing any of the copper arsenites. It may be stated that similar experiments previous and subsequent to the series of tests here reported, utilizing the 4 vegetable oils and the 3 aphid repellents and aphicides mentioned above in various combinations, but not containing any of the copper arsenites, showed that such ingredients did not cause injury to the plant tissues when employed at the same strengths and under similar conditions.

EFFECTS OF WOUND DRESSINGS UPON CANKERS

On August 2, 2 to 3 weeks after application of the wound dressings, it was found that rather severe injury occurred around the cankers to which the 3 dressings containing copper fatty-acid arsenites had been applied. The bark surrounding the cut-out or cleaned-out cankers was darkened, and in many cases sunken or depressed, particularly at the ends of the cankers (Fig. 1, A). The necrosis was characteristic of perennial canker extension as it normally occurs in bark tissue during the spring following sub-zero winter temperatures.

Detailed examination of the necrotic area revealed a discolored outer and inner bark, characteristic of tissue recently invaded by *Gloeosporium perennans.* A distinct cork layer at the edge of or within the necrotic bark

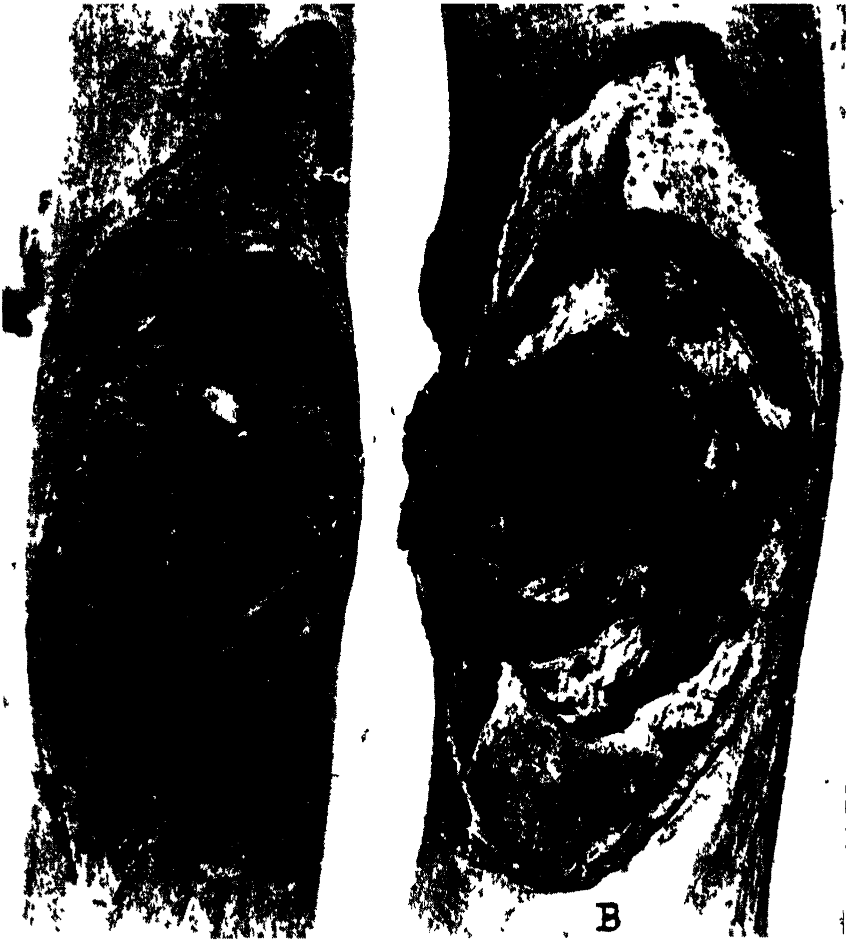

FIG. 1. A. Perennial canker on Jonathan apple limb, cleaned and painted, showing the distinct extension at each end of the canker and also at the sides, and the separation of healthy bark from that invaded by the fungus. A. Acervuli of the fungus formed after the extension had taken place and appearing as pimples beneath the thin epidermis. B. Typical canker on a Jonathan apple limb. b. The area indicated shows the bark invaded by the fungus during one spring extension period. Slightly reduced.

area was formed by the host. This formation of a cork wall by the host, which may be termed a wound response, develops in the tissue well in advance of the invading fungus. After a distinct cork wall is formed the mycelium then invades the walled-off area in from one to several days, depending on temperature, moisture, and possibly other factors. Many examples were found where several cork walls had formed, each successive wall being distinctly in advance of and formed after or near the time the fungus mycelium invaded the walled-off area. Beyond the injured bark area and along the new xylem tissues a narrow dark-brown streak was observed. This is characteristic of the advance of the fungus mycelium into such tissues.

CULTURAL AND CHEMICAL TESTS

After thoroughly sterilizing the bark around several representative cankers to eliminate surface infection, a large number of tissue plantings were made on potato-dextrose agar. *Gloeosporium perennans* was isolated from all the newly discolored bark areas around the cankers and from the dark-brown streak in the xylem extending from 1 to 3½ inches beyond the necrotic bark area. Tissue plantings from apparently normal bark or wood remained sterile.

Both injured and uninjured tissue around cankers were analyzed for arsenic. In obtaining samples for analyses, the surface bark was first removed to eliminate the arsenical spray deposit. An average content of 256 parts per million of As_2O_3 (on dry basis) was found when the entire newly discolored bark around several cankers was analyzed. Chemical analyses of apparently normal uninjured bark showed only a trace of As_2O_3.

PERCENTAGE OF CANKERS INJURED

Final examination of the cankers on the 5 Jonathan trees used in this series of wound-dressing experiments was made in October, 1932. Injury was obtained around all the cankers on 2 trees where palmito or lauro arsenites of copper were present in the dressing. The percentage of cankers on all 5 trees showing injury was as follows:

1. Wound dressing containing copper stearo arsenite	63.4
2. Wound dressing containing copper palmito arsenite	91.1
3. Wound dressing containing copper lauro arsenite	88.9

Three other dressings applied to cankers on the same trees, but not containing arsenites or other possible fungicides, showed no injury. Unpainted cankers were also left on each tree and were without injury. It should be mentioned that the above percentages do not necessarily indicate the comparative injurious nature of these 3 copper arsenites to apple tissue because different vegetable oils were used in each of the 3 dressings. Tung oil was used in wound dressing No. 1, mentioned above, perilla oil in No. 2, and soybean oil in No. 3. The movement of the arsenites into the plant tissues might have been influenced by the penetrating qualities of each oil, by the temperature when the dressings were applied, by the general consistency of the dressing, and possibly by other factors.

UNUSUAL DEVELOPMENT OF THE FUNGUS

Figure 1, B shows a typical canker on a Jonathan apple limb as it occurs under field conditions. The area indicated by b is the extension of the fungus in the bark, which, under average field conditions in North Central Washington, occurs once a year during a relatively short period at about the time tree growth starts. The extent of the bark area that may be invaded by the fungus during the early spring extension period has been shown to be correlated with the severity of the weather of the preceding winter (4, 5).

Figure 1, A is representative of a typical canker on a Jonathan apple limb, as used in the experiment, which has been cleaned and painted, as previously described. The bark area invaded by the fungus at each end of the canker and also at the sides is evident and the separation of healthy bark from that invaded by the fungus is clearly discernible.

Only a small percentage of the total number of injured cankers was cultured to prove the presence of the perennial canker fungus. Acervuli, however, formed beneath the thin epidermis covering the necrotic areas around the majority of injured cankers (Fig. 1, Aa). The unusual feature is that the fungus advanced and fruited on the injured tissue during the hottest season of the year, at a time when it is extremely difficult to obtain successful artificial inoculations and when, under ordinary circumstances, the fungus is most inactive. It was likewise interesting to note that the fungus advanced into tissue containing a total average of 256 parts per million As_2O_3. The chemical analyses, however, do not necessarily prove that such a concentration of As_2O_3 actually existed in all tissues invaded by the fungus.

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RECOVERY FROM AND ACQUIRED TOLERANCE OF CURLY TOP IN NICOTIANA TABACUM

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(Accepted for publication Feb. 13, 1939)

INTRODUCTION

Numerous reports have been made of recovery of plants from virus diseases. However, in only a very few cases have investigations been made on the recovered plants to determine if virus is still present in the plants, whether it has been changed in virulence, and whether the recovered plants have acquired any resistance to reinoculation with the same or related viruses. Wingard[1] first demonstrated acquired resistance to reinoculation in plants that recovered from a virus disease. Working with ring spot of tobacco, Wingard found that the juice of plants that had recovered from ring spot produced typical symptoms when used to inoculate healthy plants, but recovered plants were unaffected by reinoculation. Price[2, 3] made an extensive study of recovery and "acquired immunity" from ring spot in tobacco. His results fully corroborated those of Wingard and showed further that the virus was still present in plants of the tenth vegetative generation.

Lesley and Wallace[4] found that certain plants of wild races of tomatoes frequently recover, at least partially, from curly top. The virus of curly top, apparently unchanged in virulence, was present in the recovered plants. Plants grown from cuttings from recovered plants were unaffected by reinoculations with curly-top virus, while cuttings from healthy plants were seriously injured.

Recovery from and acquired tolerance of curly top in *Nicotiana tabacum* has been reported.[5] It is the purpose of this paper to report in detail the results of this study. The term "acquired tolerance" is used in this connection in preference to the term "acquired immunity." Although the latter has been used by phytopathologists in recent reports of studies on virus diseases, this usage is objectionable in that it infers a true acquired immunity in plants comparable to that in animals. The existence of such an immunity in plants has not been satisfactorily demonstrated. It seems, however, that recovery of plants from virus diseases and the acquired tolerance that sometimes accompanies recovery offer promising material for further study of the mechanics of acquired tolerance in such plants. The nature of this type of acquired tolerance, or so-called acquired immunity in plants must be understood before it can be stated with certainty that true acquired immunity does or does not exist in these plants.

RECOVERY FROM CURLY TOP IN NICOTIANA TABACUM VAR. TURKISH

Turkish tobacco can be infected quite easily by viruliferous beet leaf hoppers and infected plants usually develop severe symptoms following inoculation. Symptoms consist of dwarfed, curled leaves on the terminal growth, which tends to produce more or less of a rosette effect. Growth is retarded but, eventually, the plants recover by producing new growth that appears more or less normal. Recovery takes place either in a renewal of growth from the severely diseased terminal with the production of progressively less severely diseased leaves, or else new shoots arise from axillary buds below the terminal. If by the latter process, the new shoots appear almost normal from the beginning, showing only slight or mild curly-top symptoms. These mild symptoms consist of faint vein clearing, which is sometimes so inconspicuous that the shoot appears normal or disease-free. The same shoot may at other times show more definite symptoms, but there is very little effect on growth and flower production Recovered plants have been grown for several months, showing symptoms of slightly varying degrees, but in no case have they been observed to relapse or develop symptoms as severe as shown before recovery took place.

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[2] Price, W. C. Acquired immunity to ring spot in Nicotiana. Contrib. Boyce Thompson Inst. 4: 359–403. 1932.

[3] Price, W. C. Virus concentration in relation to acquired immunity from tobacco ring spot. Phytopath. 26: 503–529. 1936.

[4] Lesley, J. W., and J. M. Wallace. Acquired tolerance to curly top in the tomato. Phytopath. 28: 548–553. 1938.

[5] Wallace, J. M. Acquired tolerance of curly top in *Nicotiana tabacum*. (Abstract) Phytopath. 28: 674. 1938.

If inoculations are made of very young tobacco plants they are sometimes killed. However, if the plants remain alive, even though severely injured by curly top, they always seem to recover. Figure 1 shows 4 Turkish tobacco seedling plants of the same age. Plants 1 and 2 were inoculated with curly-top virus by caging 20 viruliferous beet leaf hoppers on each. Plants 3 and 4 were noninoculated controls. The plants averaged about 6 inches in height when inoculations were made and the photograph in figure 1 was made 34 days after inoculation. Plants 1 and 2 are shown in figure 2 as they appeared 64 days after inoculation. Plant 2 was dead and only its dried remains were left. On the other hand, plant 1 had produced from below the severely diseased terminal portion an axillary shoot of almost normal appearance. Figure 3 shows this recovered plant 108 days after inoculation. The dead, dried original diseased shoot is shown on the rim of the pot. The first recovered shoot is flowering and another quite normal-appearing basal shoot has developed Frequently, the only obvious symptoms on such shoots as the large one of figure 3 are swellings or papillae-like enlargements on the calices.

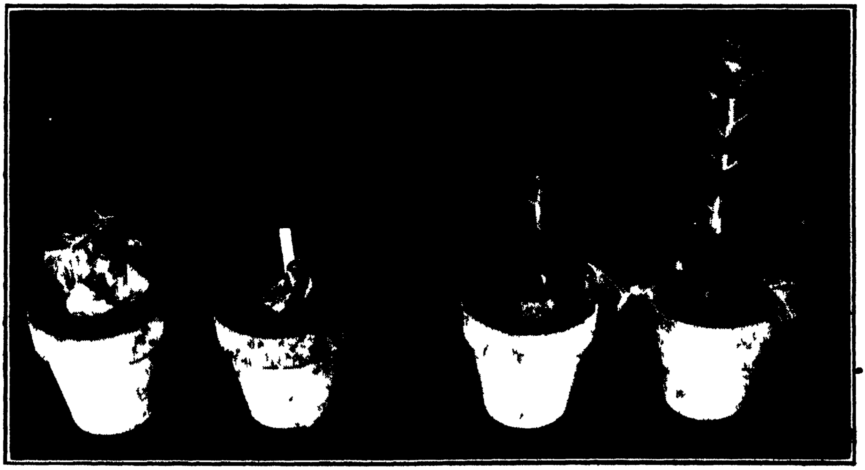

FIG. 1. Curly top on *Nicotiana tabacum* plants. Plants 1 and 2 inoculated by leaf hoppers. Plants 3 and 4 noninoculated controls. Photographed 34 days after inoculation.

VIRULENCE OF VIRUS IN RECOVERED TOBACCO PLANTS

Transmission of virus from recovered plants to healthy tobacco plants directly by means of beet leaf hoppers resulted in typically severe curly-top symptoms on the healthy plants. Also, when non-viruliferous leaf hoppers were fed on solutions containing an extract of the alcoholic precipitate of juice from recovered plants and then caged on healthy sugar beets, the resulting infections produced severe symptoms. Transmission from these beets to tobacco produced severe symptoms on tobacco. It is evident from these tests that the virus in recovered plants was unchanged in virulence, so far as tobacco and beets are concerned.

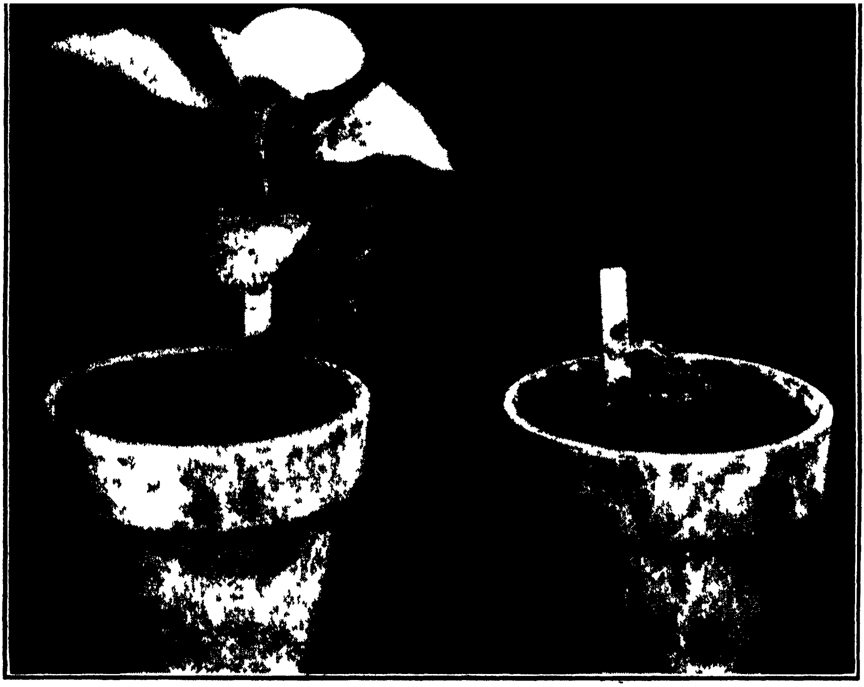

FIG. 2. Plants 1 and 2 of figure 1, 64 days after inoculation. Plant 1 shows recovery by the production of a normal appearing basal shoot.

ACQUIRED TOLERANCE IN RECOVERED PLANTS

Plants grown from cuttings from recovered plants showed curly-top symptoms of varying degrees of mildness. Some of the plants were sometimes free of symptoms, while at other times they showed definite symptoms. Cuttings from healthy, curly-top-free plants usually rooted sooner than those from recovered plants and usually grew somewhat faster, particularly during the early stages. The first leaves formed from axillary buds of cuttings from recovered plants sometimes showed fairly severe symptoms for a short time. This happened most frequently when the cuttings failed to produce roots or were slow in doing so. However, as the rate of growth increased, the symptoms became less severe and the mild type then prevailed. Symptoms were sometimes more severe on the early leaves of the first axillary shoots that developed after a recovered plant had been cut back.

Figure 4, A, shows cuttings grown from healthy and recovered plants. All of the plants in figure 4, A, were inoculated on the day this photograph was taken. Fifteen viruliferous leaf hoppers were caged on each plant. None of the cuttings from recovered plants showed any effect of reinoculation, while 5 of the 6 cuttings from healthy plants developed severe curly top. Figure 4, B, shows two plants from each group 28 days after inoculation.

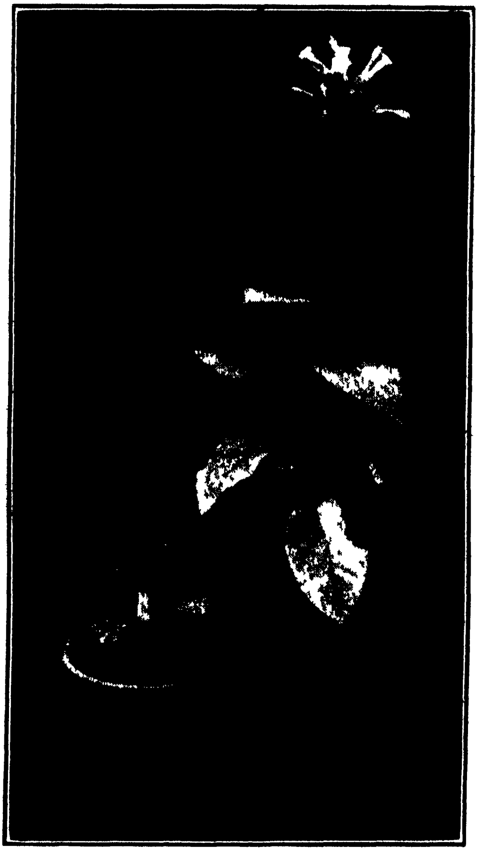

FIG. 3. Plant 1 of figures 1 and 2, 108 days after inoculation. The dead, dried remains of the original severely diseased terminal shoot are shown on the rim of the pot. The first shoot to appear after recovery has flowered quite normally and a second basal shoot has developed.

In a total of 52 recovered plants reinoculated, there has been no visible effect. In some of the tests as many as 30 viruliferous leaf hoppers per plant were used for inoculation. A high percentage of the healthy check plants became infected, and these all developed typically severe symptoms.

Recovery from one strain of curly-top virus gave protection against a second strain. Furthermore, recovery from infection with a single strain conferred protection against subsequent inoculations with groups of leaf hoppers from mixed viruliferous stock colonies. It has not yet been determined whether strains other than the one from which the plants recover can become established in the recovered plants.

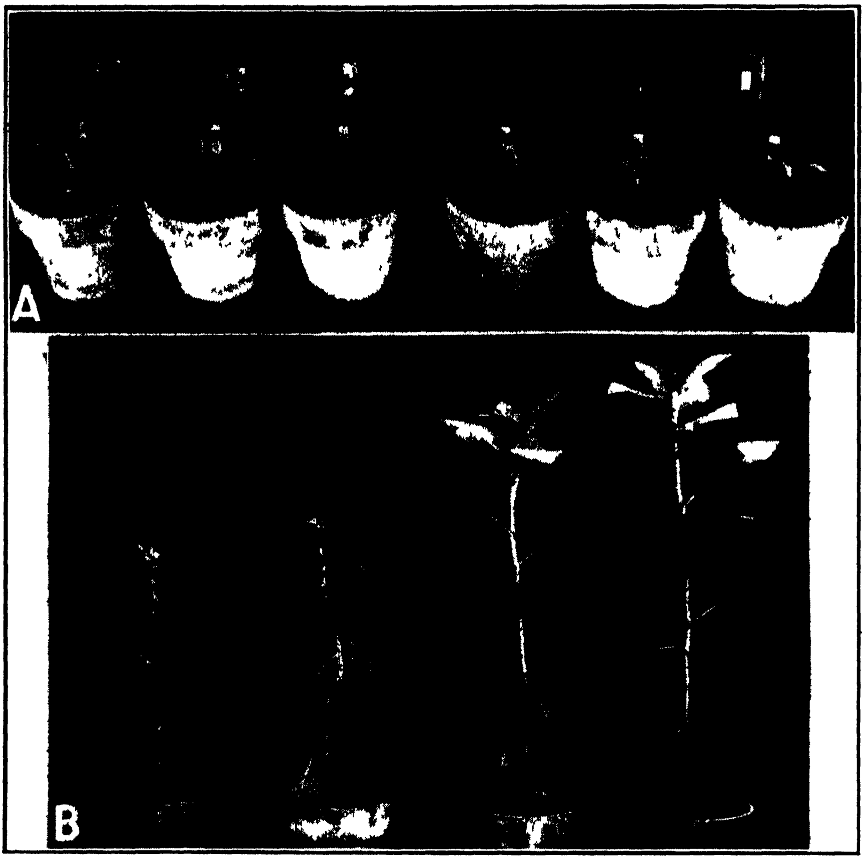

FIG. 4. Acquired tolerance in cuttings grown from recovered tobacco plants. A. 6 plants from cuttings from healthy plant on left and 6 cuttings from recovered plant on right at time of inoculation. B. 2 plants from each corresponding group after exposure to viruliferous leaf hoppers.

Curly-top virus was still present in plants of the fourth vegetative generation and such plants showed the same degree of tolerance as was shown in the first generation following recovery.

SUMMARY AND CONCLUSIONS

Nicotiana tabacum plants commonly recover from severe symptoms of curly top by the gradual production of less severely diseased leaves from the severely diseased terminal, or by the production of axillary shoots, free of symptoms, or nearly so. Plants grown from cuttings from recovered plants show a slight variation in symptom expression from time to time, but in no instance have they relapsed permanently into a stage equalling the typical symptoms produced by inoculation of healthy plants by beet leaf hoppers.

Virus is present in recovered plants and was found in plants of the fourth vegetative generation.

Cuttings grown from recovered plants showed no effects when reinoculated with curly-top virus.

Recovery from one strain of curly-top virus conferred protection against another strain as well as against mixed, unidentified strains of the virus.

Recovery of tobacco from curly top and the acquired tolerance associated with it are similar in many respects to those reported for ring spot of tobacco and curly top of tomato. The suggestion is made that acquired tolerance following recovery of plants from virus diseases may offer the best material yet available for studying the nature of immunological-like phenomena in plants.

BOX 752, RIVERSIDE, CALIFORNIA.

REPORT OF THE 1939 ANNUAL MEETING OF THE SOUTHERN DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The 1939 annual meeting of the Southern Division of The American Phytopathological Society was held in connection with the meeting of the Association of Southern Agricultural Workers on February 1–3, inclusive, in New Orleans, Louisiana. About 35 formal papers were given and several periods were devoted to the discussion of general problems, including seedling diseases and Fusarium wilt and Phymatotrichum root rot of cotton. While most of the papers dealt with cotton diseases, a substantial number related to diseases of other crops. The average attendance at the four sessions was approximately 55.

The afternoon of February 2 was spent on a tour to the Freeport Sulphur Mines, located near New Orleans.

A short business session was held on the morning of February 3, when the following officers were elected:

President, A. G. Plakidas
Vice-president, C. H. Arndt

Titles and abstracts of papers presented at the meeting follow.

LUTHER SHAW
Secretary-Treasurer

Leaf Blotch, A New Disease of Rice and Certain Native Plants in Louisiana. T. C. RYKER. A hitherto unreported disease of rice was observed in a field near Crowley, Louisiana, in July, 1936. Most rice plants along one levee appeared as if they had been sprayed with some caustic material. The leaves and leaf sheaths showed large, irregular, bleached areas with deep reddish-brown margins. These spots coalesced, resulting in the death of a large portion of the leaf tissue. There was a reddish cast to the bleached areas. In some instances the spots were small, scattered, and of quite circular outline. Many weeds in the area were similarly affected. Notable among these were *Caperonia castaneaefolia, Axonopus furcatus, Echinochloa crusgalli,* and *Paspalum spp.* The disease has been found since on rice in a number of fields and on Bermuda grass, *Cynodon dactylon,* in the southwest Prairie, on carpet grass, *Axonopus compressus,* near Beaumont, Texas, and on a sedge, *Carex frankii,* near Baton Rouge, Louisiana. Invariably, the diseased tissue has yielded a sterile fungus of distinct cultural behavior. It grows extremely rapidly and produces thin, grayish-brown, stromatic crusts in and on the substratum, suggesting a species of *Ciboria* or of a related genus. All attempts to induce fruiting have failed. Moreover, a fruiting stage has not been observed in nature. The disease has been reported on rice by placing bits of the fungus mycelium in contact with leaf and leaf-sheath tissue.

Further Studies on Control of Soil Rot of Sweet Potatoes. L. H. PERSON. To study further the effect of sulphur on control of soil rot of sweet potatoes, extensive field tests were made using 4 commercial brands of sulphur. Because of unfavorable planting and growing conditions, somewhat inconsistent results were obtained. However, in those fields where conditions most nearly approached those of a normal season, very promising results were obtained. Applications of 600 to 800 lb. of sulphur per acre lowered the pH from 5.8–6.0 to 4.8–5.0. In the sulphured plots growth was apparently normal, while in the control plots the plants remained small, became chlorotic, and many of them died. Yields in heavily infested soils were increased from almost nothing in the control plots to 75–115 crates of U. S. 1 and 2, field run, in the sulphured plots. Preliminary results obtained from a portion of the field sulphured in 1937 (without further application) indicated that the sulphur was effective for more than a 1-year period.

The Necessity of Rotation of Crops for the Control of Diseases of the Sweet Potato. R. F. POOLE. Since the sweet potato is used as a vegetable in the South, it is grown on many soil types. *Fusarium batatatis* causes serious wilt on Norfolk, Durham, Sassafras, and Granville soils. Heavy losses on the other soil types are rarely seen. Experimental tests indicate that the organism may not live well in soil types where the disease is rarely seen. Continuous growth of sweet potatoes on soils favorable to the development of the disease greatly increases the inoculum. *Heterodera radicicola* causes heavy losses on Norfolk, Durham, and Mallboro soils, and continuous planting on these soils resulted in greater losses. *Ceratostomella fimbriata* occurs on all soil types, and appears to live for many years in the absence of the sweet potato. The use of infested lands for other crops is suggested. *Monilochaetes infuscans* occurs on all soils but is most prevalent on heavy soils. It is shown that the fungus does not live in the soil for an extended period, and 3 years' rotation with other crops greatly reduces the losses from this disease.

Seed and Soil Treatments for Combating Damping-off of Tomatoes, Eggplants, and Peppers. L. H. PERSON. Experiments were made over a 4-year period in which a number of seed and soil treatments were tested for their effectiveness in controlling damping-off of tomato, eggplant, and pepper seedlings. The predominating fungi causing damping-off in this area belong chiefly to the genera *Rhizoctonia* and *Pythium*. Numerous isolations made from diseased seedlings grown in naturally infested soil gave 10 *Rhizoctonia* isolates to one of *Pythium*. Red copper oxide (cuprous oxide), Metrox, and Semesan were used as fungicides. Various commercial formaldehyde dusts and a concentrated formaldehyde solution were used as soil treatments. The dusts were applied at the rate of 1½ oz. per sq. ft. of soil area. Zinc oxide was used as a combined soil and seed fungicide, applied at the rate of 20 g. per sq. ft. at the time of seedling emergence. Over a 4-year period red copper oxide proved to be the most effective seed fungicide for tomatoes and peppers. The effectiveness of this material was primarily attributable to its control of the preemergence phase of damping-off. For eggplant, red copper oxide supplemented with a zinc oxide soil fungicide at time of seedling emergence proved the most effective treatment. Concentrated formaldehyde was an effective soil fungicide if precautions were taken against recontamination. Formaldehyde dusts gave inconsistent results; and in some tests Semesan, used as a seed fungicide, caused a marked reduction in germination. In general, for tomato and pepper, seed treatment should give adequate protection, while for eggplant it alone is inadequate.

The Possible Control of Root-rot Fungi by Soil Treatment with Chemicals. R. F. POOLE. This paper gives a general treatment of the substances produced by microorganisms and the effects of these substances on plant parts. It is shown that certain necrotic effects may result from organism activities in the soil medium without parasitic expression. It is indicated that changes made in the soil hydrogen and hydroxyl ions offer very limited values in the control of the most prevalent parasitic organisms in the soils of North Carolina. Wide changes in the soil reactions maintained over a period of 5 years have been ineffective in bringing about control by biological means. It also appears that most of the soils in North Carolina are sufficiently acid to inhibit the parasitism of *Thielaviopis basicola* and *Actinomyces scabies*.

Difference in Susceptibility of Tomato Varieties to Septoria and Macrosporium Leaf Spots. J. O. ANDES. Resistance to the two common leaf spots varies among different varieties of the common tomato. Intensification of this character is being attempted by crossing plants within and between the more promising lines. Considerable variation in other characters also appears in the progenies. Segregates are being crossed for combinations of all desirable characters.

Control of Cercospora Leaf Spots of Peanut with Various Dusts and Sprays. **LUTHER SHAW.** The value of sulphur dust in control of Cercospora leaf spots of peanut was measured in North Carolina in 1937 and 1938. Three applications were made to each of 12 1-acre plots, at as many separate locations in 1937, and to 15 locations in 1938. The first application was made about July 25, the second about August 14, and the third about September 1, each season. Approximately 16 pounds of sulphur was applied per acre at each application, and reduced the prevalence of leaf-spot lesions on peanut leaflets approximately 75 per cent each season. The amount of defoliation resulting from leaf-spot infection was reduced about 70 per cent each season. The average increase in yield of peanuts per acre on the dusted plots when compared with similar nondusted ones was 343 pounds in 1937 and 217 pounds in 1938. Substantial increases in hay yields were obtained each year. In replicated 1/40-acre test plots, at 2 locations in 1938, 3 applications of 4–4–50 Bordeaux mixture, 1½ to 50 cuprocide 54 and sulphur dust gave excellent control of leaf spot and substantial increases in the yields of peanuts and hay. One to 40 liquid lime sulphur gave good control of the disease but injured the foliage, and, therefore, did not give a marked increase in yield. Pyrethrum dust showed no fungicidal value and gave only a slight increase in yield of peanuts and no increase in yield of hay.

Bordeaux Injury to Cucumbers. **A. G. PLAKIDAS.** Field tests for the control of downy mildew of cucumbers (*Pseudoperonospora cubensis*) were made in the fall of 1938, with 4–4–50 Bordeaux, "Copper Spray 34," and "Copox" dust. Satisfactory control was obtained with both sprays. The dust gave a fair degree of control, but was less effective than the sprays. The 4–4–50 Bordeaux severely injured the cucumber plants. The injury was of 3 types: (1) Severe stunting of the growth of the vines; (2) marginal burning of the young leaves; and (3) burning ("scorching") of the localized, irregular areas on the older leaves. The Bordeaux injury was reflected in the yields. In 3 replications, the plots sprayed with "Copper Spray 34" gave 27.7 per cent, 31.6 per cent, and 93.4 per cent higher yields, respectively, than the corresponding plots sprayed with 4–4–50 Bordeaux.

Carbon Dioxide Evolution from Certain Soils in Relation to Black Root Rot of Flue-cured Tobacco. **J. A. PINCKARD** and **LUBEN BOZOVAISKY.** Samples of 600 g. each of Granville sandy loam and Cecil sandy loam soil types (Chatham, Virginia), adjusted to ⅔ of their moisture holding capacities, were placed in respiration chambers for various periods of time, at stated temperatures, for volumetric estimation of carbon dioxide. Estimations were made, almost daily, following field transplanting of tobacco and until 2 months after harvest. *Thielaviopsis basicola* was a limiting factor of growth in the Cecil sandy-loam soil type (pH 6.2) and was absent from the Granville sandy-loam type (pH 5.7). Triplicate samples from around the roots of plants in field plots receiving 800 lb. of a 3–10–6 fertilizer mixture, and from adjacent unfertilized check plots, showed little differences in 7 samplings made through the season. Carbon dioxide production, per sample, per day, ranged between 2 and 8 mg. After harvest, 3 g. of chopped Italian rye-grass plants, or tobacco plants, were incorporated in the samples. Almost complete decomposition of these residues was effected within 30 days. The low yields of carbon dioxide in these 2 tobacco soils indicate a minimum total microbial population and a minimum of decomposable organic matter regardless of the presence of *Thielaviopsis basicola*.

An Internal Collar Rot on Cotton. **C. J. KING** and **H. D. BARKER.** A hitherto unreported root rot of cotton has been observed in the vicinity of Sacaton, Arizona, for several years. It recurs year after year in the same areas, causes heavy mortality to seedlings when the soil is cold and wet, and is largely inactive during hot weather, resuming activity to destroy more plants in late summer. Infected tissues show a purplish-black discoloration, and microscopic examination reveals in the vessels abundant dark brown, many-segmented chlamydospores of a fungus. In pure culture this fungus produces also endogenous cylindrical spores and in all morphological characters it appears to be identical with *Thielaviopsis basicola*, cause of the black-root disease of tobacco. In inoculation experiments this fungus has produced all symptoms except mortality of mature plants and has been recovered in cultures. American-Egyptian varieties are more susceptible than upland varieties. The fungus persists in the soil for years, but does not spread rapidly and is, therefore, not especially threatening as a cotton-disease factor.

Root-knot Nematodes on Cotton and Tomatoes in Tennessee. **C. D. SHERBAKOFF.** In 1938, on a 100 × 120 foot plot at Knoxville, Tennessee, no root knot was found on varieties of upland cotton following tomatoes that has been severely damaged by *Heterodera marioni*. The same year, considerable root-knot injury was observed on 12 varieties of upland cotton, in a large part of the plot used for regional cotton-wilt studies, at Tiptonville, Tennessee. The plot at Knoxville was used for tomato culture continuously for 12 years, and the plot at Tiptonville for cotton culture for a much longer time. It is suggested, as a working hypothesis, that the host specialization of the nemas is due primarily to the segregation and survival of favored genetic races.

Observations on the Root-knot Nematode in the San Joaquin Valley of California. C. E. SCOTT, M. A. LINDSAY, and GEORGE J. HARRISON. The root-knot nematode is observed to be a serious pest of several important crops, particularly Acala cotton, in the San Joaquin Valley. It is present on all weeds and grasses in certain districts and is sometimes accompanied by the meadow nematode. Where nematodes are well-established, rotation of cotton with alfalfa and barley is proving disappointing as a means of control. Until recent years these rotations have been regarded favorably. After 2 years of fallow, cotton grew satisfactorily for a year on heavily infested soil, but a susceptible bean variety was killed within 8 weeks after planting. Nematode galls are found in soil at a depth of 28 inches below the surface, which may explain why ordinary fallow is not a wholly effective control. In pot experiments involving controls, infected, infected air-dry, and infected steam sterilized soils, cotton grew well in the controls, infected soil produced numerous galls, and air-drying and steam sterilization successfully controlled nematodes, but retarded plant growth. Inoculation of clean soil with pure culture of root-knot nematodes was unsuccessful. When mixed with infected soil, undecomposed manure gave good plant growth in early summer but the effects soon were exhausted.

Preliminary Report on Cotton Wilt-nematode Experiments at Lumberton, North Carolina. A. L. TAYLOR, H. D. BARKER, and O. P. OWENS. Previous field observations have indicated that a severe infestation of root knot nematodes may lessen the effectiveness of certain wilt resistant varieties of cotton. The meadow nematode also has recently been observed to cause considerable injury to cotton under field conditions. Such observations, however, have not been sufficiently extensive to evaluate the extent of injury or the possible relationship to the incidence of wilt. The failure of a wilt-resistant variety at Lumberton in 1937 was attributed to a heavy soil infestation of *Fusarium vasinfectum*, root-knot nematodes, and meadow nematodes and offered an excellent opportunity for experimental study of relationships under field conditions of natural infestation. Ten varieties, ranging from tolerant to highly wilt-resistant, were included in combinations with 2 rates of potash application in carbon bisulphide-treated and nontreated plots. All varieties in the treated plots gave moderately good growth. In the nontreated, relatively few plants survived. This treatment appeared to be effective against the 2 types of nematodes, although rather rapid reinfestation led to some complications for determining wilt and nematode relationship. It did not appear to have damaged the wilt organism. Varietal responses were not so distinct as had been hoped for, although encouraging differentiations were suggested.

Benefits of Winter Green-manure Crops in Controlling Phymatotrichum Root Rot of Cotton. C. J. KING and J. T. PRESLEY. In a field experiment in progress for 3 years at the U. S. Field Station, Sacaton, Arizona, 6 kinds of winter green-manure crops gave beneficial effects in controlling Phymatotrichum root rot when double-cropped with cotton. These, with average percentages of dead cotton areas after 3 double crops, were as follows: Bard Vetch, 7; Canada field peas, 10; sour clover, 2; Trieste mustard, 11; and wild mallow, 5. Rye, the only graminaceous crop included, gave on 2 plots an average of 42 per cent of area affected by root rot compared to 34 per cent on control plot. These data, although preliminary, support previous conclusions that drastic modification of the soil microflora by the deep application of liberal quantities of organic materials may produce soil conditions unfavorable for the parasitic root-rot fungus. Results with green manures suggest that residues from graminaceous crops may be less effective in control than those from legumes and certain nonlegumes that are high in nitrogen. In this and other experiments the incidence and extent of root-rot infestation shows a relationship to the abundance of the microfloral populations. Delayed mortality of plants and low infestation usually is associated with high total organism count in the soil and early and extensive infestation with low organism count.

Relation of Variations in Rainfall in 1938 to Prevalence of Cotton Root Rot. WALTER N. EZEKIEL. A series of 1,590 estimates in cotton fields in 28 counties in Texas was used in a study of the effect of weather conditions on local prevalence of Phymatotrichum root rot. Results of multiple curvilinear correlation analyses agree with those of 1937 in showing that destructiveness of root rot on cotton within the favorable blackland-prairies soil areas was limited largely by rainfall. As in 1937, the effect of a given increase in rainfall was progressively greater for the later months of the season. With the higher rainfall in 1938, however, it was possible to demonstrate inflexion points beyond which additional increase in rainfall did not increase the percentage of root rot. For percentages of plants killed by October 5–15, 1938, these inflexion points were: June, about 4 inches of rainfall; July, about 3 inches; and September (rainfall mostly in first 12–14 days), about 2 inches. Up to these points, the final percentage of root rot was apparently almost exactly a linear function of the rainfall during the several preceding months, with the slopes of the lines for the several months increasing with lateness in the season. It may be possible to set up estimating equations by which weather data can be used to estimate probable prevalence of root rot, or experimental results secured under varying rainfall conditions reduced to more nearly comparable figures.

Girdling of Cotton Plants as Affecting Survival of Phymatotrichum omnivorum. WALTER N. EZEKIEL. A possible new point of attack against the root-rot fungus on cotton and other host plants (suggested by recent work of R. Leach with *Armillaria mellea*) was tested in preliminary experiments with cotton plants. Plants girdled on July 29, 1938, showed bronzing of foliage within 11 days. Within a month a third of the plants were dead. Meanwhile, there was rapid decrease of alcohol-soluble solids and total sugars in the roots. There was no viable *Phymatotrichum omnivorum* on roots excavated after 3, 5, and 8 weeks from girdled plants, either those that had root rot at the beginning of the experiment or adjoining plants to which root rot ordinarily would have spread; while the fungus grew readily from roots from nontreated (control) plants. Another experiment was started on September 3. At this time girdling produced no apparent change in aboveground appearance of cotton plants, even after a month; nor was the viability of *P. omnivorum* reduced on roots of girdled plants or of plants with tops cut off. In this test, girdling of cotton stems during the period of rapid growth in summer apparently made the roots no longer suitable for continued survival or spread of the root-rot fungus, while similar treatment in fall did not cause such change.

Attempts to Control Verticillium Wilt of Cotton and Breeding for Resistance. B. A. RUDOLPH and GEORGE J. HARRISON. Verticillium wilt was first reported on cotton in the San Joaquin Valley in 1930 by Rudolph and Shapovalov, and observed on the United States Cotton Field Station, Shafter, in 1929, but not so identified. A record of its spread on the Station has been kept. This shows that the heavier soils that have been cropped to cotton during the last 10 years have become severely infested, and some are totally so. The spread of the disease usually has been against the flow of irrigation water. It is not established in light sandy soils. Anhydrous ammonia, anhydrous ammonia plus ammonium polysulphide cyanamide, and soil sulphur were used on infested plots 3 consecutive years, but have failed to control Verticillium wilt of cotton. Also, sulphate of ammonia and carbon bisulphide failed in 1938. Infestation of the breeding plots has been maintained by annual artificial inoculation. By selection, fairly resistant strains of Cook 307–6, Mexican Big Boll, Kekchi, Tuxtla, and Missdel have been isolated. Although not resistant, strains of Stoneville and Acala yield well under heavy infection. American-Egyptian cottons are highly resistant to *Verticillium*. Pima is being used in a program of back-crossing with Acala in an attempt to transfer Pima resistance to Acala.

Permeability of the Testa of Normal and Treated Cotton Seeds. J. G. BROWN. Normal, fuzzy cotton seeds sprout erratically when planted in the field and in the germinator; a greater or lesser percentage of the viable seeds exhibit delayed germination for days or even weeks. Acid-treated seeds of the same lot sprout promptly. Studies of the seeds afford an explanation: (a) air-bubbles entrapped in the lint of normal cotton seeds and in contact with the seed-coat reduce the water-absorbing surface; (b) the seed-coat is a selective or semipermeable membrane through which water passes more rapidly after acid-treatment. The air-carrying capacity of the lint on cotton seeds has been measured. The studies on the permeability of the cotton seed were made with an osmometer adapted for holding a piece of the seed-coat, thus eliminating possible factors involving other parts of the seed. Water intake through both normal and acid-delinted seed-coats is increased as the temperature rises, suggesting that the seed-coat, as well as the embryo, is affected by the temperature of the soil at time of planting. The imbibitional "pull" of cotton seeds is strong enough to absorb water from a saturated salt (NaCl) solution and from a saturated solution of lithium chloride.

Fungi Associated with Seedling Diseases and Boll Rots of Cotton in Eastern United States in 1938. PAUL R. MILLER. Surveys in which 14 State and Federal pathologists cooperated to obtain information regarding the prevalence and relative distribution of fungi associated with damping-off of seedlings and boll rots of cotton in 1938 demonstrated that *Glomerella gossypii* was the most widely distributed and predominant organism on both diseased seedlings and bolls. It predominated on seedlings from all States (South Carolina, Georgia, Alabama, Mississippi, Tennessee, Louisiana, Texas, North Carolina, and Virginia) surveyed, except Texas, being isolated 283 times from a total of

344 samples, whereas *Fusarium moniliforme* and *Rhizoctonia solani* were recovered 239 and 44 times, respectively. *G. gossypii* was the most prevalent organism on culture plates 240 times out of the total 344; *F. moniliforme* and *R. solani* 14 and 11 times, respectively. Other fungi isolated in order of descending frequency were: *Fusarium* spp., *Pythium* spp., *Diplodia gossypina, Rhizoctonia bataticola, Fusarium vasinfectum, Sclerotium rolfsii,* and *Aspergillus* spp. The wide distribution and the frequency of *G. gossypii* occurring on bolls represented a sequel to the seedling survey results. It was found 133 times out of 141 fields sampled. *Fusarium moniliforme* occurred less frequently than *Glomerella*. Only *Alternaria* spp. appeared more often. *Fusarium* sp., *Diplodia gossypina,* and some undetermined fungi also were isolated.

Factors Influencing the Distribution and Persistence of Angular Leaf Spot in Irrigated Cotton Fields. C. J. KING and R. B. PARKER. In cotton-seedling-disease experiments conducted at the U. S. Field Station, Sacaton, Arizona, for 2 seasons, seed of the Pima and Acala varieties, after delinting with sulphuric acid or treatment with 2 per cent Ceresan, showed no early angular-leaf-spot infection. Later, however, rows and plots of seedlings that were free from the disease after such seed treatments became infected, apparently, from irrigation water that, at higher levels, had submerged infected seedlings. Areas of disease-free seedlings, irrigated directly from the distributing ditch until early summer, when the cotyledon leaves had shed, remained free from angular leaf spot throughout the period of seedling development. Recurrence of angular-leaf-spot epidemics in some commercial areas, in spite of precautions to plant supposedly disease-free seed, were attributed to infection spread by early irrigations from diseased volunteer seedlings. As precautions to avoid extensive recurrence in diseased areas, it is suggested that cotton-plant residues should be raked and burned, disease-free seed should be planted, early cultivations should be made to destroy volunteer seedlings, and waste water and run-off from other cotton fields should be diverted.

Effect of Period and Type of Storage of Cotton Seed After Treatment with Organic Mercury Dusts. L. E. MILES. Cotton seed, 1936 crop, D and PL 11 A, stored 1, 2, 3, 4, and 5 months after treatment with Ceresan, 3 oz. per bu., and New Improved Ceresan, 2 oz. per bu., in dry storage in the laboratory and in ventilated corn crib in open, showed, in 1937, on statistical analysis of data, no significant difference either in emergence or yield as a result of either period or type of storage. The same was true in 1938 after 17 months' storage after treatment. The effect of the treatments themselves, irrespective of type of storage or period of storage, up to 17 months after treatment, the limit in this test, was highly significant. In 1937, after periods of storage up to 5 months, the average increase in emergence for seed treated with Ceresan over the nontreated control was 25.07 per cent. For New Improved Ceresan, 24.61 per cent. In 1938, after 17 months storage after treatment, increases in emergence for Ceresan and New Improved Ceresan, respectively, were 24.11 per cent and 38.85 per cent. In 1937, after storage up to 5 months, increases in yield over control were, Ceresan, 24.5 per cent, New Improved Ceresan, 26.43 per cent. In 1938, after 17 months storage, they were, Ceresan 27.85 per cent, New Improved Ceresan, 35.83 per cent. This test shows that seed may be treated any time after harvest with organic mercury dusts and stored for periods up to 17 months without injurious effects as a result of treatment and without decreasing the beneficial effects of the treatment.

Seed-treatment Tests with Cotton in 1938. D. C. NEAL. Seed-treatment tests with cotton involving 3 seed lots: fuzzy, mechanically delinted, and sulphuric acid-delinted, and 4 dust treatments: Ceresan, Improved Ceresan, Cuprocide, and Barbak C, were conducted at 2 localities in Louisiana in 1938, with 2 dates of planting at each locality. Data were collected and analyzed for seedling emergence, stem lesions, and yield in experiments at Baton Rouge and for emergence and yield at St. Joseph, Louisiana. In the first planting at Baton Rouge none of the seed lots or dust treatments gave differences in emergence that were significant (1 per cent point); while in the later planting all dust treatments and the mechanically delinted stock gave significant increases. Also, all dusts and both delinted lots in that planting yielded seedlings that were significantly lower in stem lesions caused by anthracnose, *Rhizoctonia,* and *Fusarium* spp. At St. Joseph, in the early planting, the increases in emergence were highly significant for all dusts except Barbak C; while in seed lots, the sulphuric acid seed gave a significant reduction. In the later planting at that locality, only Improved Ceresan of the dust treatments significantly increased emergence; while of the seed lots, the mechanically delinted was significant. The differences in emergence resulting from the dust treatments and seed lots in the 2 planting dates at both localities are thought to be attributable to weather conditions. Increases in emergence were not reflected in higher yields at either locality.

Seedling Survival as Affected by Certain Mercury, Copper, and Zinc Preparations not Included in the Regional Cotton Seed Treatment Tests for 1938. S. G. LEHMAN. One- and 4-year-old cotton seed, dusted with the commercial preparations Ceresan (2 per cent ethyl mercury chloride), Sanoseed (2 per cent ethanol mercury chloride), and Sterocide (4 per cent mercury furfuramide diluted to 2 per cent with talc) was planted on April 22 and May 5 in Norfolk fine sandy-loam soil. In both early and late plantings the increases in seedling survival from use of Ceresan were highly significant statistically, the average increase being 49.7 per cent for the 2 dates of planting. In the early planting, Sterocide and Sanoseed increased the stand by 16.2 and 15.7 per cent, respectively, but these increases only approach statistical significance. In the later planting both these preparations gave highly significant increases, 24.5 and 23.0 per cent, respectively. The average for both dates of planting also shows highly significant stand increases for both these materials. At both dates of planting the increase resulting from Ceresan was significantly greater than that induced by either Sterocide or Sanoseed. Ceresan gave approximately equal gains with 1- and 4-year-old seed. Sterocide and Sanoseed gave larger increases with 1-year-old seed. In another test, in which seed dusted with AAZ Special zinc oxide, Cuprocide (commercial preparation), Copper oxychloride, and cuprous oxide were compared with nontreated seed at 2 dates of planting, only zinc oxide increased the percentage of seedling survival significantly. This increase was small and probably not economically important.

Treatment of Cotton Seed with Organic Mercury Dust and Sulphuric Acid. GEORGE J. HARRISON. From 1936 to 1938, inclusive, experiments have been conducted at the U. S. Cotton Field Station, Shafter, California, with the treatment of cotton planting seed with organic mercury dust and with sulphuric acid delinting. Organic mercury has added materially to the stands of cotton when temperatures are unfavorable for germination; acid delinting has shown no advantage over no treatment at all; and delinted seed with organic mercury added showed no advantage over organic mercury alone. Neither the use of organic mercury nor of sulphuric acid delinting has given any significant increase in yields of cotton. After seedling emergence is complete, neither has given any control over seedling diseases such as rhizoctonia.

Variability of Fusarium vasinfectum in Culture. R. WEINDLING. Thirteen monosporous isolates of *Fusarium vasinfectum* from cotton were used. They had been kept in culture for periods ranging from 5 months to 6 years. Development of variants was studied by frequent subculturing on potato-dextrose agar slants from which monosporous isolates were taken to Petri dishes for comparison after each transfer. Eleven isolates developed variants that tended to dominate the parent type. The time interval at which the respective variants arose was not constant. The behavior of the organism agreed favorably in the following respects with that of *Fusaria* studied by other investigators: (1) all original isolates from diseased plants were of "type A" (combining rapid radial growth and development of abundant aerial mycelium); (2) cultural changes were primarily in the direction of decreased aerial and radial growth; and (3) all "type A" cultures were highly pathogenic; some variants were highly pathogenic but most of them were not.

Some Tests of Varietal Susceptibility to a Combination of Nematodes and Cotton Wilt. L. E. MILES. A test with 17 varieties of upland cotton and 14 varieties and strains of introduced and hybrid cottons was conducted in 1938 on land heavily infested with both wilt and nematodes. One-row plots, 25 feet in length were used with 8 replications. Of the upland varieties, a selfed line of Missdel No. 4 showed 100 per cent infection with both nematodes and wilt, with 85 per cent of plants dead at harvest and a yield of 375 pounds of seed cotton per acre. Other upland varieties varied in infection with wilt between 14 per cent for Clevewilt 6 to 72.5 per cent for Half & Half, and with nematodes from 45.63 per cent for Clevewilt 6 to 75.25 per cent for Washington. Yield varied from 1600 pounds of seed cotton per acre for Dixie Triumph 12, 1550 for Clevewilt 6, to 400 pounds for Half & Half. Of the foreign and hybrid cottons a strain of Hopi showed 100 per cent of plants infected with both wilt and nematodes, all dead, and no open bolls. Sea Island 13B3 showed no infection with wilt and lowest, 44.5 per cent, with nematodes. Tuxtlal, Kekchi, Sakel, and Sea Island were most resistant to wilt than most upland varieties, and all but Sea Island 13B3 were more susceptible to nematodes than most upland varieties.

Artificial Inoculation with the Cotton-wilt Fungus, Fusarium vasinfectum. D. C. NEAL. Artificial inoculations involving 2 soil types and 4 varieties of cotton, Delfos 965-425, Dixie Triumph-12, Clevewilt 6, and Half & Half, were continued in the greenhouse in 1938 for the purpose of developing an accurate wilt-infection technique suitable for rapid testing of new strains and varieties for wilt resistance. The soil types employed were (1) Lintonia silt loam, mixed with equal parts of leaf mold and sand; and (2) Ruston sandy loam, both being maintained at 60 per cent of their water-holding capacity during growth of the plants. Inoculum consisted of bean pod and Czapek's solution cultures of the fungus. Results were obtained indicating that a variety may be safely classed as resistant if infection does not exceed 15 to 20 per cent during a growth period of 45 days or less in soil artificially inoculated. When grown in presence of both wilt (*Fusarium vasinfectum*) and nematodes (*Heterodera marioni*) in Ruston sandy loam soil for a period of 44 days, all of the 4 varieties developed a high wilt infection. These results conform largely to their behavior under similar field conditions.

Progress in Soil-contamination Studies with Fusarium vasinfectum. A. L. SMITH. The establishment of desirable breeding plots in the proximity of experiment stations often necessitates the development of epidemic disease conditions. Studies here reported are attempts to produce high wilt infestations comparable to those occurring naturally in isolated locations. Earlier results indicated the possibility of using an oat-wheat mixture that could be produced in relatively large quantities, air-dried, and stored until needed. More recent studies indicate the following conclusions: A reduction in infected plants occurred the second year following contamination. Recontamination resulted in a considerable increase in infected plants over the first contamination. No significant difference in amount of wilt resulted from the application of comparable amounts of air-dried and freshly prepared contaminant. The percentage of infected plants increased directly with increased amounts of contaminant up to 750 pounds per acre. Grinding to a finely divided condition resulted in a significant increase in the amount of wilt compared with the whole grain material.

Relation of Unbalanced Fertilization to the Fusarium Wilt of Cotton. V. H. YOUNG and W. H. THARP. Using an arrangement of field plots allowing analysis of variance, a series of what are commonly considered balanced and unbalanced fertilizers were applied to cotton on a sandy alluvial soil in eastern Arkansas. The land possessed suitable amounts of available phosphorus and nitrogen but was highly deficient in potash. Cotton wilt and potash hunger, both in severe form, had been previously noted. In 1937 fertilizer applications resulted in few significant differences in the amount of cotton wilt with respect to the checks, but high-potash plots showed significantly less wilt than low potash-high phosphate plots. In 1938, potash fertilization more effectively controlled wilt; in addition, the application of unbalanced fertilizers, high in phosphorus but lacking potash, gave significant increases in wilt. Balanced fertilizers had a highly beneficial effect upon yields in both years. Although the effects of potash fertilization on cotton-wilt control were most apparent when highly susceptible Half & Half cotton was used, the combined effects of varietal resistance to wilt and the beneficial effects of balanced fertilization in controlling both potash hunger and wilt resulted in appreciably greater yields for the wilt-resistant varieties Rowden 2088 and Cook.

The Effects of Nitrogen Source, Nitrogen Level, and Relative Acidity on Fusarium Wilt of Cotton. W. H. THARP and C. H. WADLEIGH. Factorially designed, greenhouse sand-nutrient experiments were employed to study the association of variations in level of nitrate and ammonia nitrogen (20 and 100 ppm.) and variations in pH of the solutions (4, 6, and 8), with relative wilt severity in resistant, tolerant, and susceptible cotton varieties. The analysis of variance showed a highly significant increase in wilt severity associated with increase in nitrogen level and a significantly greater severity associated with the ammonia source. The variance of wilt severity resultant from change in pH was not consistently significant as a main effect (all treatment-variety combinations considered) but pH was shown to be a significant contributor to the variance of the interaction with nitrogen source. Susceptible plants, supplied with the high nitrate solution at pH 8 were *less* severely diseased than at either pH 6 or pH 4, while susceptible plants supplied with high *ammonia* solutions at pH 8 were more severely diseased than at either pH 6 or pH 4.

Effects of Nitrogen, Phosphorus, and Potassium Nutrition on the Fusarium Wilt of Cotton. W. H. THARP and C. H. WADLEIGH. Sand-nutrient-culture infection technique was employed to study the relation of variations in nitrogen (4, 20, and 100 ppm.), phosphorus (1/10 and 30 ppm.), and potassium (2 and 40 ppm.) associated with relative wilt severity in resistant (Rhyne Cook), tolerant (Rowden 2088), and susceptible (Half & Half) cottons. The results from the factorially designed experiments show statistically significant differences in wilt severity associated with variations in level of each of the 3 elements studied. Increase of nitrogen and phosphorus levels was associated with significant increases in wilt severity, while increase in potassium level was accompanied by a significant reduction in severity. A study of the interactions of the treatment-variety combinations showed that the effect of many of the nutrient combinations was not the same for all 3 varieties. The disease severity in resistant and susceptible varieties was nearly proportionally affected by treatment, while severity in the tolerant cotton was, in many treatments, affected disproportionately with the other two varieties. Similarly, the disease severity associated with variations in level of one element was not always proportionally the same at all variations in level of the other two elements.

A Study of Virulence in Relation to Cultures of Fusarium vasinfectum. E. M. CRALLEY. Half & Half and Rhyne's Cook cotton, a susceptible and a resistant variety, respectively, were inoculated with 80 separate cultures of *Fusarium vasinfectum* collected from various parts of Arkansas. The results show that there is a wide range in the virulence of the cultures; however, in all instances Half & Half was more susceptible than Rhyne's Cook. Using the sand culture technique, an experiment was conducted to determine the response, as indicated by the amount of wilt obtained, of a mildly, moderately, and very virulent culture to various nutrient solutions. The experiment was set up so that different levels of nitrogen, phosphorus, and potassium could be combined in various combinations. The results show that in the case of the cultures employed, even though the amount of wilt obtained varied greatly with the type of nutrient solution used, the relative virulence of the cultures in all cases remained essentially constant.

PHYTOPATHOLOGICAL NOTES

Hybridization of a Mosaic-tolerant, Wilt-resistant Lycopersicon hirsutum with Lycopersicon esculentum.—In the collection of wild tomato species made by H. L. Blood in South America in 1937–38, for the Division of Plant Exploration and Introduction, there were several samples of a species of *Lycopersicon* that have been identified by C. H. Muller as *L. hirsutum* H. and B. All of these appear to be of one uniform type and under Beltsville, Maryland, field conditions produce large, luxuriant vines that flower profusely in late summer and set a few fruits. The abundant glandular hairs secrete an oily substance that has a strong pungent odor, slightly suggestive of catnip. The fruits are pubescent, round-oblate, 1 to 1½ cm. in equatorial diameter and when mature are greenish ivory in color with medium dark green radial strippings (Fig. 1, E). They are two-loculate, with thin, tough walls. The pulp is watery and contains numerous small straw-color seeds.

In the summer of 1938, crosses were made between *Lycopersicon hirsutum* and two commercial varieties of *L. esculentum* Mill., Marglobe and Bonny Best. Pollen from field-grown plants of *L. hirsutum* was used to pollinate emasculated blossoms of Marglobe and Bonny Best growing in a screened greenhouse. Immediately after pollination these blossoms were covered with glassine bags until the fruits were partially developed. Seed from these fruits germinated readily, and the first-generation hybrid has been grown. The dominance of the *L. hirsutum* foliage characters is outstanding in the F_1 generation (Fig. 1, B). The fruit is globular (Fig. 1, D, F) and somewhat larger than that of *L. hirsutum*, being 2 to 2½ cm. in diameter. The epidermis is yellow-pigmented and the interior walls and pulp a paler yellow with a mildly subacid, fruity flavor, somewhat similar to that of *L. esculentum* fruits, the outer and inner wall being much heavier and more succulent than that of *L. hirsutum*. The fruits thus far produced have been two-loculate, with 6 to 10 fully developed seeds. A few seeds have been germinated and young F_2 plants are now growing. Backcrosses to *L. esculentum* have produced an ample amount of viable seed. In the greenhouse the F_1 hybrid is self-fertile and cross-fertile on the *L. esculentum* parents; the *L. esculen-*

tum pollen, however, has thus far failed to fertilize either the flowers of the first generation hybrid or those of *L. hirsutum*. On the other hand, *L. hirsutum* pollen readily fertilizes *L. esculentum* flowers and also those of the first-generation hybrid.

This hybrid is of particular interest because it appears that *Lycopersicon hirsutum* is highly tolerant to tobacco-mosaic virus and resistant to Fusarium wilt (*F. bulbigenum* var. *lycopersici* Wr. and R.). In 1938 plants of *L. hirsutum* proved extremely resistant to Fusarium wilt when grown in the field on heavily infested soil. These plants also showed no evidence of mosaic infection, although the disease was prevalent on other wild and cultivated tomatoes in adjacent rows.

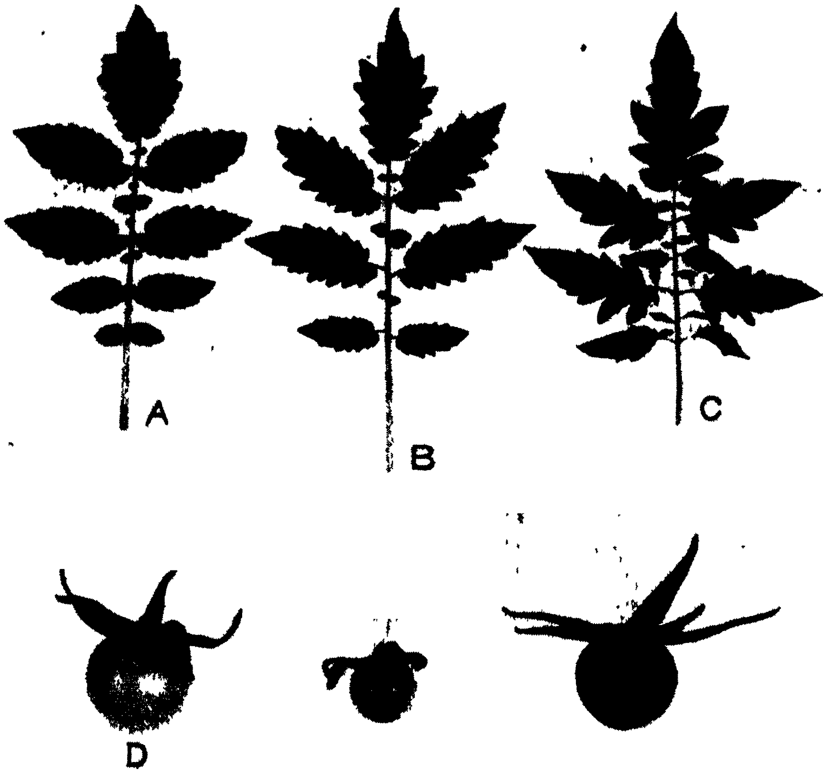

FIG. 1. Leaflets from 8-week-old plants of *Lycopersicon hirsutum*, A; *L. esculentum*, Marglobe, C; and F_1 hybrid leaflet, B. ⅓ natural size. Fruit of *L. hirsutum*, E; and F_1 hybrid fruits: *L. hirsutum* on Bonny Best, D; and *L. hirsutum* on Marglobe, F. ⅔ natural size.

Further tests of the reaction of *Lycopersicon hirsutum* to mosaic were made in the greenhouse on eleven rooted cuttings from healthy plants inoculated with a strain of tobacco virus 1, Johnson, which produces particularly severe symptoms on tomatoes. During the 4 weeks following the inoculations both the inoculated and checks plants made an equally vigorous growth. The inoculated plants showed no vein clearing, mottling, necrosis, or other symptom that would in any way distinguish them from the checks. At the end of 5 weeks, inoculations were made from all the plants to Turkish tobacco, and 100 per cent infection was obtained from all of those inoculated. The 5 check plants proved mosaic-free. Further inoculations with juice from the infected *L. hirsutum* plants to *N. glutinosa* showed a high virus concentration in all the inoculated plants, which was somewhat unexpected, since plants of some mosaic-tolerant tobaccos show a low concentration of tobacco virus 1, and the same is true of cucumbers tolerant to cucumber mosaic (cucumber virus 1). The apparently complete tolerance of *L. hirsutum* to tobacco mosaic is of especial interest because of the fact that no species or variety of *Lycopersicon* previously tested by the writers has failed to show at least mild symptoms of mosaic.—W. S. PORTE, S. P. DOOLITTLE, and F. L. WELLMAN, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

Penetration of Potato-tuber Tissue by Rhizoctonia solani in Relation to the Effectiveness of Seed Treatment.—Seed potato treatment for the control of *Rhizoctonia* is generally practiced in Aroostook County, Maine. The mercuric chloride 1–1000, 1½ hour-soak method is commonly used, but many growers use the more rapid dip methods and the acidulated mercuric chloride treatment. A high percentage of stem and tuber infection in fields grown from treated seed potatoes indicated that these materials were ineffective or that infection resulted from soil infestation.

Seed pieces that had been treated with the above disinfectants were collected from growers' fields immediately after planting and the sclerotia were cultured. Many of the large sclerotia proved viable, and in many cases the tuber tissue directly under the larger sclerotia produced the *Rhizoctonia* fungus in artificial culture. Tests were made for viable mycelium in periderm tissue under large sclerotia that had been treated in 1–1000 mercuric chloride for 2 hours in the laboratory. After thorough washing in tap water and rinsing in sterile distilled water the sclerotia were removed along with the cork cells directly under them. The periderm tissue thus uncovered was removed with a sterile needle and transferred to culture medium in Petri dishes. Eleven of the 32 cultures developed *Rhizoctonia.* After removing the adhering cork tissue the sclerotia were cultured, but none produced growth in nutrient agar.

The presence of viable mycelium of *Rhizoctonia* under a non-viable sclerotium indicated the possibility that the mycelium penetrated the periderm tissue of the tuber to a depth sufficient to make it inaccessible to disinfectants. With this in mind, sclerotia with underlying tuber tissue were fixed, sectioned, and stained with Orange-G and thionan. The results obtained are shown in figure 1. Figure 1, A and B, represents sections through the center of a small sclerotium and the underlying tissue. The mycelium has penetrated but has not gone through the cork layer, only a few of the cork cells having been invaded C and D show cross sections through a larger sclerotium overlying a lenticel The mycelium has invaded several cell layers, and is mostly intercellular. In E and F the mycelium has penetrated the layer of cork cells, passed through the cork cambium, and entered the tissue below the periderm This mycelium probably gained entrance though a lenticel

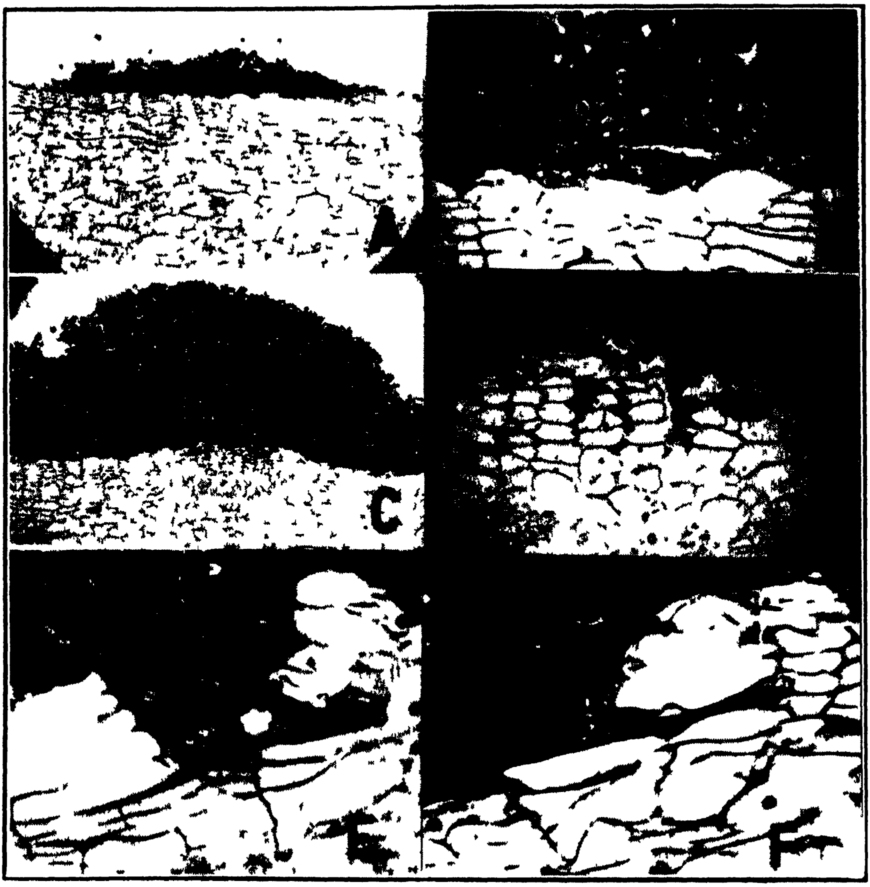

FIG. 1. Cross sections through sclerotia growing on the surface of potato tubers. A and B Through a small sclerotium, B is a higher magnification of A and shows a slight invasion of the cork cells. C and D Through a large sclerotium: note lenticel invaded by the fungus mycelium E and F. Mycelium extending to and below the periderm.

Invasion of the periderm and the tissue below may offer sufficient protection to the mycelium to prevent complete killing by seed disinfectants. This might account for in some Rhizoctonia infection from treated seed potatoes—L A SCHAAL, Bureau of Plant Industry, U S Department of Agriculture, Washington, D. C

A Permanent Transferable Culture-tube Label.—The tin-backed cardboard (tagboard) label, as now manufactured (Fig. 1), is a split cylinder, 1¼ inches long and 2 inches in circumference. A ⅛-inch, metal edge with 45-degree bevelled corners is folded outward over the face of the cardboard. The label slips easily over the ends of tubes of various sizes and grips them tightly. When transferred from a larger to a smaller tube, the edges of the label need simply be sprung together before slipping it onto the smaller tube to make it grip tightly. The cardboard writing surface, which is of a grade permitting use of ink without feathering, is large enough for adequate labeling. Dates of transfers can be written in pencil on the label and later erased or kept in a card index. A Public Service patent for this label was applied for on November 23, 1938. The Fisher Scientific Company is already manufacturing it.

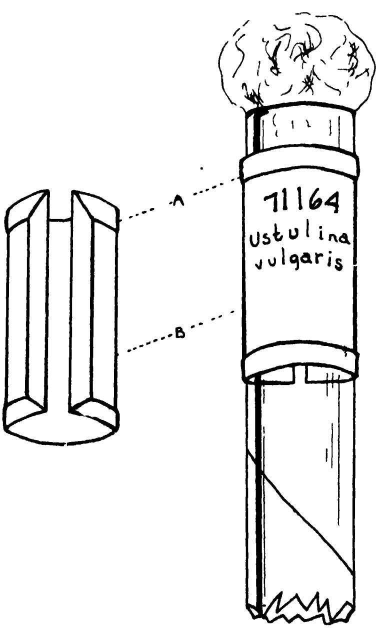

FIG. 1. Diagram of permanent transferable label: A, Tin; B, cardboard surface.

Any thin, light, sufficiently resilient metal may be substituted for the tin. The writing and metal can be protected by a clear lacquer.

Once the label is written, or printed, it remains as a permanent identification for the culture during the entire period of its maintenance through numerous transfers. Slipping the label off the old tube and onto the new one in which the transfer is made eliminates the need of rewriting the data on new labels. It should, therefore, prove to be a great time-saving device in the maintenance of large collections of stock cultures. It also should eliminate either the possibility of error in writing many sets of labels for the same culture or the loss of gummed paper labels.

The need for a permanent, transferable test-tube label was first suggested by L. O. Overholts of the Pennsylvania State College to W. A. Campbell of the Division of Forest Pathology. Because of their suggestions and encouragement and the very apparent need of such a label in maintaining a large collection of cultures of wood-destroying fungi in the Division of Forest Pathology, the writer designed the permanent transferable label for stock cultures of fungi and bacteria.—DOROTHY J. BLAISDELL, Civilian Conservation Corps, Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

EFFECT OF CROWN AND STEM RUSTS ON THE RELATIVE COLD RESISTANCE OF VARIETIES AND SELECTIONS OF OATS[1]

H. C. MURPHY[2]

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INTRODUCTION

Although it is generally recognized that winter injury of fall-sown oats is mostly a direct or indirect result of freezing temperatures, it is apparent also that several factors affect the ability of a particular variety to withstand a specific exposure to such temperatures. A variety may be injured or killed under one environment, while under a different one the same exposure may cause little or no injury. Among the factors possibly affecting the ability of oat varieties to acquire winter hardiness are light conditions during the hardening period and the presence of crown rust (*Puccinia coronata avenae* Eriks. and Henn.) and stem rust (*P. graminis avenae* Eriks. and Henn.) infection. Dexter (2, 3) has shown that winter-wheat plants acquire and retain winter hardiness more readily when maintained at a cold temperature in light than when maintained at the same temperature in darkness. He found that, in general, the hardening and maintenance of the hardened condition are favored by conditions tending toward accumulation or conservation of organic food reserves; that is, conditions that favor photosynthesis but with reduced vegetative growth. Mains (6) found that Trumbull winter wheat, initially inoculated with leaf rust on September 20 and heavily infected by November 13, showed a survival of 4.1 per cent in comparison with 77.7 per cent for rust-free plants. The writer (10, 11) has shown that heavy infection of crown rust on oats, in addition to reducing the yield of all plant parts and increasing water requirement, results also in a great decrease in the sugar content of the green plants.

Autumnal infection of fall-sown oats by crown rust may occur whenever inoculum is available and weather conditions are favorable. Such infection is often severe on early fall-sown oats in the South Central and Southeastern states. While severe autumnal infection with stem rust is observed less frequently, it also may occur under favorable conditions.

The present study was undertaken to determine the effects, if any, of crown- and stem-rust infections on cold resistance of oats and on the relative resistance of different varieties and selections. Approximately 120 varieties and selections of winter oats were sown in the field in the autumns of 1933, 1934, and 1936 at Ames, Iowa, and the seedling plants of one series inoculated with crown rust. None of these survived; therefore, all the data here presented were obtained from investigations carried on under greenhouse conditions at Ames in the winters of 1933–34 and 1934–35. Preliminary reports of the data here presented have been published by the writer (7, 9).

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[1] Journal Paper No. J-595 of Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 72. Cooperative investigation between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Botany and Plant Pathology Section of the Iowa Agricultural Experiment Station.

[2] The writer gratefully acknowledges indebtedness to W. E. Loomis of the Botany Department, Iowa State College, for helpful suggestions and criticisms during the course of the experiments; to T. R. Stanton and F. A. Coffman of the Bureau of Plant Industry, U. S. Department of Agriculture, for furnishing seed of the standard winter oat varieties; and to N. I. Hancock, of the Tennessee Agricultural Experiment Station, for supplying seed of the Tennessee winter oat selections.

MATERIAL AND METHODS

For a preliminary study of the effect of crown rust on cold resistance, made in the fall of 1933–34, the following were included: 26 varieties, grown in the 1933–34 uniform oat winter-hardiness nurseries maintained by the Division of Cereal Crops and Diseases, United States Department of Agriculture, in cooperation with the agricultural experiment stations of certain States; 35 winter-oat varieties and selections supplied by the agricultural experiment stations of Tennessee, Georgia, and Texas; and 266 varieties previously studied for crown-rust resistance by the writer and listed in table 14 of a recent publication (8). Although satisfactory differentiation was obtained, the number of varieties was too large for continuation in the more detailed experiments.

In subsequent studies 30 varieties were used for 6 experiments conducted during the winter of 1933–34, and 40 varieties and selections for 5 experiments in the winter of 1934–35. Twenty-one varieties were common to both seasons. These groups of 30, 40, and 21 varieties and selections are listed in tables 4, 5, and 6, respectively. The varieties and selections included were those outstanding for resistance to cold in the preliminary tests, and additional varieties of special interest, such as Victoria (C. I.[3] 2401), Bond (C. I. 2733), Iogold (C. I. 2329), and Markton (C. I. 2053), which were, respectively, highly resistant to race 1 of crown rust; nearly immune from race 1 of crown rust; highly resistant to race 2 of stem rust; and completely susceptible to both rusts. Glabrota (C. I. 2630) and *Avena brevis* (C. I. 1783) were included because they were the most susceptible to injury from cold of any of the 327 varieties and selections tested in the preliminary study.

In the preliminary studies the first year the plants were grown and frozen in flats and, subsequently, in 4-inch pots. Sufficient seed was planted to allow thinning to 5 plants per pot. The plants were grown in a greenhouse where the temperature was automatically controlled at 60° to 65° F. until the winter types reached the rosette stage of development with 3 to 7 tillers per plant. At this stage, except as indicated otherwise, the plants in part of the pots were inoculated with race 1 of crown rust or race 2 of stem rust by dusting a mixture of urediospores and talcum powder onto the previously moistened plants with a small hand duster. The inoculated plants were kept in a moist chamber in the 60–65 degree house for 18 hours following inoculation. Then all the inoculated and control plants of a particular experiment were moved to a greenhouse unit maintained at 38° to 42°, where the plants were hardened for 10 to 21 days, depending upon the experiment. The temperature controls did not include artificial cooling and, for this reason, all experiments were conducted during the winter when outside temperatures ordinarily were low enough to allow a greenhouse temperature of 42° F. Whenever outside temperatures became too high the plants being hardened were removed to a lighted refrigerator room maintained at a constant temperature of 40°. Hardening by exposure to near freezing or gradually decreasing temperatures seems to more nearly approach normal field conditions, even though drought, nutrition, etc. also may be important factors affecting hardening. Apparently, most conditions that check the vegetative growth of oat plants but allow for continued photosynthesis will increase their resistance to cold.

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[3] C. I. refers to accession number of the Division of Cereal Crops and Diseases formerly Office of Cereal Investigations.

In one experiment an attempt was made to determine the effect of sodium nitrate and sodium chloride on relative cold resistance and also whether response to these salts might be related to hardiness. In these experiments the plants were watered with concentrations of 1, 2, 5, and 10 per cent of the two salts when placed in the hardening room. Fifty cc. of solution were added to each pot at each watering, the interval between waterings depending on weather conditions. In 3 experiments plants were shaded for periods of 10 and 14 days with 1 and 2 layers of gauze during hardening to determine whether the supposed shading effect of rust spores could have any effect on cold resistance. In one of these experiments plants infected with crown rust were frozen when chlorotic flecks appeared but before any urediospores had developed that could cause shading.

Plants were frozen by placing them in freezing rooms maintained at constant temperatures either of 20° or 0° F. Most of the plants were exposed at 20° for 24 hours, although some were kept at this temperature for 48 and others for 20 hours. Another exposure at 0° for 1 to 4 hours sometimes was used. The room maintained at 20° had a floor space of 9 × 10 feet and was 9 feet high with freezing coils suspended from the ceiling. This was large enough to allow all the plants to be exposed at floor level. The room maintained at 0° was too small to permit all pots being placed on the same level, so a fan was used to circulate the air. All plants were uniformly watered approximately 4 hours before being placed in the freezing chamber.

Following exposure to freezing temperatures the plants were returned to the 40° F. room. Preliminary studies indicated that plants maintained at 40° for a period of 5 to 10 days after being frozen recuperated in a manner more nearly approximating that occurring under field conditions than did plants returned immediately to 60° to 65° temperatures. Records on plant condition were taken 5 days after freezing. The relative injury resulting from exposure to freezing temperatures was determined by the method described by Quisenberry (13), in which both the estimate of frozen tissue and the percentage survival are taken into account (Table 1). If all 5 plants in a pot were uninjured the hardiness index for the pot was 5 × 20 or 100, whereas if all plants were very weak the hardiness index of the pot would be 5 × 4 or 20. Intermediate indices also were recorded, that is, values of 2, 6, 10, 14, and 18 for individual plants, following the criteria presented in table 1.

TABLE 1.—*Criteria for evaluating plant condition 5 days after exposure to freezing temperatures*[a]

Description	Visible criteria	Assigned numerical value
Dead	No sign of life	0
Very weak	Showing only small amounts of living tissue	4
Weak	Leaves more than half killed	8
Heavy injury	Leaves about half killed	12
Light injury	Tips of leaves killed	16
No injury	No sign of killing	20

[a] After Quisenberry (13).

At intervals during the investigation plants were retained 30 days in the 38° to 42° F. greenhouse room, following exposure to freezing temperatures and their percentage survival recorded. Although there is a high positive correlation between the hardiness index determined 5 days after exposure and the percentage survival 30 days after exposure, the former appeared to give the more accurate measure of the relative cold resistance of the material tested in these preliminary studies. Plants retained at 38° to 42° for 30 days, following freezing, often died, apparently as a result of secondary effects rather than because of exposure to low temperatures, *i.e.*, plants showing only slight injury 5 days after freezing would sometimes turn yellow and die, while other plants showing much more injury would survive. Another and more serious objection to using the 30-day percentage survival is the fact that it limits observations to two broad classes of plants, *i.e.*, living and dead, whereas the 5-day evaluation allows observation of at least 6 distinct gradients of plant condition. For greenhouse studies where space and time often are limiting factors the advantage of the 5-day evaluation is obvious.

RESULTS

Effect of Rust Infection on Cold Resistance

During the two winters 1,530 four-inch pots containing 7,650 juvenile oat plants were subjected to various treatments and exposed to freezing temperatures. The detailed results showing the effects of rust, shading, and salt solutions on hardiness are given in table 2. Within any one of the 11 experiments all seed was sown, and freezing was started, at the same time, and, unless otherwise noted, the plants were maintained under the same conditions until the five-day readings were obtained.

A 30 per cent infection of crown rust in experiment 1 lowered the hardiness of the 30 varieties approximately 38 per cent. In experiment 2, a 60 per

TABLE 2.—*The effect of crown and stem rusts on the resistance of oat varieties to low temperatures*

Experiment	Treatment	Exposure	Average 5-day hardiness index					
			30 varieties		40 varieties and selections		21 varieties[a]	
		1933–34		*Per cent*[b]		*Per cent*[b]		*Per cent*[b]
1	Watered with $NaNO_3$ (1 per cent)	20 hr., 20° F.	38.7	+37	....	....	43.8	+37
	Watered with NaCl (1 per cent)	do	37.6	+33	....	....	42.9	+34
	Control (rust-free)	do	28.3	....	....	....	31.9	....
	Crown rust (30 per cent)	do	17.6	−38	....	....	20.0	−37
2	Control (rust-free)	24 hr., 20°	84.8	....	....	....	92.3	....
	Crown rust (60 per cent)	do	35.9	−58	....	....	36.8	−60
	Check (rust-free)	48 hr., 20°	31.5	−63	....	....	35.0	−62
	do do	30 hr., 20°; 1 hr., 0°	27.5	−68	....	....	31.6	−66
	do do	48 hr., 20°; 4 hr., 0°	17.4	−80	....	....	19.5	−79
3	Control (rust-free)	24 hr., 20°	76.5	....	....	....	86.4	....
	Crown rust (30 per cent)	do	44.4	−42	....	....	50.5	−42
	Control (rust-free)	48 hr., 20°	44.3	....	....	....	53.1	....
	Crown rust (30 per cent)	do	19.7	−56	....	....	22.1	−58
4	Control (rust-free), 6 leaves	24 hr., 20°	70.2	....	....	....	83.2	....
	Crown rust (60 per cent), 6 leaves	do	24.0	−66	....	....	30.0	−64
	Control (rust-free), 6 leaves	47 hr., 20°; 1 hr., 0°	12.5	−82	....	....	16.9	−80
	do do 4 leaves	do do	1.4	−98	....	....	2.0	−98
	do do do	24 hr., 20°	58.7	−16	....	....	66.4	−20
5	Control (rust-free)	24 hr., 20°	75.4	....	....	....	87.0	....
	Crown rust (20 per cent)	do	66.0	−13	....	....	75.5	−13
	do (50 per cent)	do	39.5	−48	....	....	46.0	−47
	Control (rust-free)	48 hr., 20°	63.0	....	....	....	74.5	....
	Crown rust (20 per cent)	do	49.7	−21	....	....	58.4	−22
	do (50 per cent)	do	20.7	−67	....	....	23.6	−68

TABLE 2.—(*Continued*)

Experiment	Treatment	Exposure	Average 5-day hardiness index					
			30 varieties		40 varieties and selections		21 varieties[a]	
				Per cent[b]		*Per cent*[b]		*Per cent*[b]
		1933–34						
6	Control (rust-free)	24 hr., 20°	70.6				79.3	
	Stem rust (25 per cent)	do	57.3	-19			68.9	-13
	do (45 per cent)	do	47.1	-33			55.0	-31
		1934–35						
7	Control (rust-free)	24 hr., 20°			86.0		80.1	
	Stem rust (15 per cent)	do			77.2	-10	72.9	- 9
	do (40 per cent)	do			63.6	-26	59.3	-26
8	Control (rust-free)	do			86.1		79.4	
	Crown rust (40 per cent)[c]	do			56.8	-34	43.9	-45
	Shaded (10 days, 1 gauze)	do			48.5	-44	34.3	-57
9	Control (rust-free)	do			62.5		53.3	
	Crown rust (40 per cent)	do			38.5	-38	27.9	-48
	Stem rust (50 per cent)	do			35.4	-43	25.5	-52
	Crown rust (80 per cent)	do			22.9	-63	15.5	-71
	Shaded (14 days, 1 gauze)	do			32.8	-48	24.0	-55
10	Control (rust-free)	do			69.2		52.9	
	Crown rust (80 per cent)	do			18.6	-73	17.1	-68
	Stem rust (85 per cent)	do			10.1	-85	4.8	-91
	Shaded (14 days, 2 gauze)	do			15.1	-78	7.6	-86
11	Control (rust-free)	do			78.8		71.4	
	do do	30 hr., 20°			71.1	-10	60.2	-16
	Stem rust (60 per cent)	24 hr., 20°			37.8	-52	24.5	-66

[a] Common to both seasons and both groups of 30 and 40 varieties and selections.
[b] Percentages expressed as increase or decrease from control plants of same experiment.
[c] Infected plants showed chlorotic flecks but no uredia.

cent infection lowered the hardiness index of the same varieties 58 per cent or slightly less than did doubling the exposure period. Data from experiment 2 indicate that an added exposure of 1 hour at 0° F. caused more injury than 18 additional hours at 20°. That the effect of crown rust infection on cold resistance becomes greater with increased exposure is indicated in experiment 3 where a 30 per cent infection lowered the hardiness index of the 30 varieties 42 per cent with an exposure of 24 hours at 20° and 56 per cent with double that exposure. Increasing exposure of rust-free plants from 24 to 48 hours was almost equal in effect to a 30 per cent infection of crown rust. This effect was greater than in experiment 2, possibly because of a difference in hardening of the two groups.

The survival of the varieties Custis, Lee, Nortex, and Fulghum (winter type) (C. I. 2498), exposed 48 hours at 20° F. (experiment 3), is illustrated in figure 1. The 30 per cent infected plants of Custis and Lee were injured beyond recovery, while the rust-free plants were all alive at the end of 30 days. Both the rust-free and infected plants of the less hardy Nortex were injured beyond recovery, but with a 24-hour exposure and a 50 per cent infection (experiment 5) the reaction for Nortex was similar to that illustrated for Custis and Lee. Even the rusted plants of the more hardy Fulghum (winter type) (C. I. 2498) exposed 48 hours were not injured beyond partial recovery in experiment 3. A slightly longer exposure doubtless would have caused complete differential killing between the rust-free and infected plants of this variety.

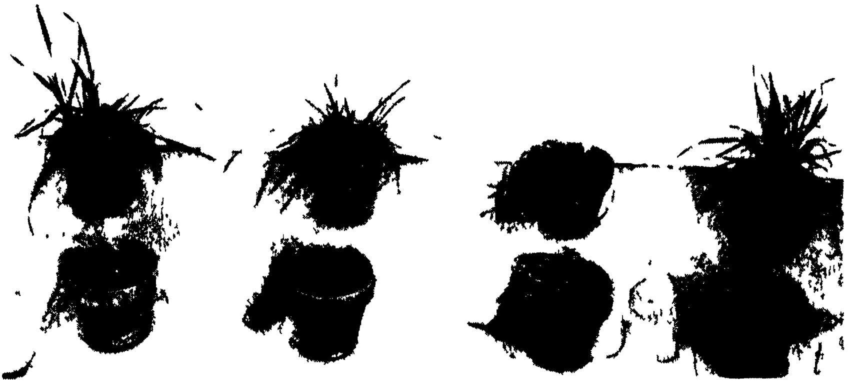

FIG. 1. Survival 30 days after an exposure of 48 hours at 20° F. *Left to right:* Custis, Lee, Nortex, and Fulghum (winter type) (C. I. 2498). *Upper row:* Rust-free plants. *Lower row:* Infected with 30 per cent severity of crown rust. (Experiment 3.)

The average hardiness index of rust-free plants with the same exposures varies considerably in the different experiments. Some of this variation doubtless is attributable to differences in maturity, although it appears likely that the greater part of the variation is traceable to differences in hardening of the plants in the different experiments brought about by differences in light intensity, length of day, and possibly slight differences in the temperature at which they were hardened. Consequently, a comparison of the relative effect of different amounts of rust infection on cold resistance can be made most accurately with plants of the same experiment inoculated and frozen simultaneously.

Some plants in experiment 5 uniformly dusted with a mixture of talcum powder and urediospores developed a 20 per cent crown-rust infection, while other plants, similarly dusted with a mixture containing a smaller proportion of talcum, developed a 50 per cent infection. The 20 per cent infection lowered the average hardiness index of the 30 varieties 13 and 21 per cent with exposures of 24 and 48 hours, while the 50 per cent infection lowered their hardiness index 48 and 67 per cent, respectively.

Stem rust was used in experiments 6 and 7. Since the average hardiness indices of the 21 varieties common to these two experiments were almost identical insofar as the effect of the 24 hours exposure to 20° F. was concerned, the results may be considered together. The estimated average stem-rust infections of 15, 25, 40, and 45 per cent lowered the hardiness index of the 21 varieties 9, 13, 26, and 31 per cent, respectively.

The plants in part of experiment 8 were subjected to freezing temperatures after an infection of crown rust had developed chlorotic flecks but before any uredia were macroscopically evident. Although the infection had not developed to the point where any shading effect from rust spores was possible, it had brought about considerable chlorosis of the leaves. This chlorotic type of infection, estimated as 40 per cent on the basis of the chlorotic areas, lowered the hardiness index of the 40 varieties 34 per cent. Shading these same varieties for 10 days with one thickness of gauze lowered their hardiness index 44 per cent. It is evident, however, that the major effect of crown-rust infection on cold resistance is operative before any shading from extraneous urediospores takes place. The differential reaction of the varieties in experiment 8 is not easily discernible in figure 2 because photographs made 5 days after exposure showed no sharp contrast between injured and noninjured tissues. Photographs made 30 days after exposure (Figs. 3, 4) were much more satisfactory, although, as previously mentioned, the 5-day reading was selected as the most representative of the actual cold resistance of the varieties.

Despite considerable variation between the different experiments in similarly exposed rust-free plants, it seems worthwhile to compare briefly the effect of different intensities of rust infection upon the cold resistance of these varieties. A summary of the effects of the different treatments on the average 5-day hardiness index of the 21 varieties used in all 11 experiments is present in table 3. It is evident that the effect of infection with either rust upon the cold resistance of hardened winter types is dependent upon the amount of infection present during the hardening period and upon the degree of exposure. There appears to be almost a direct relation between the percentage infection and percentage reduction in the hardiness index

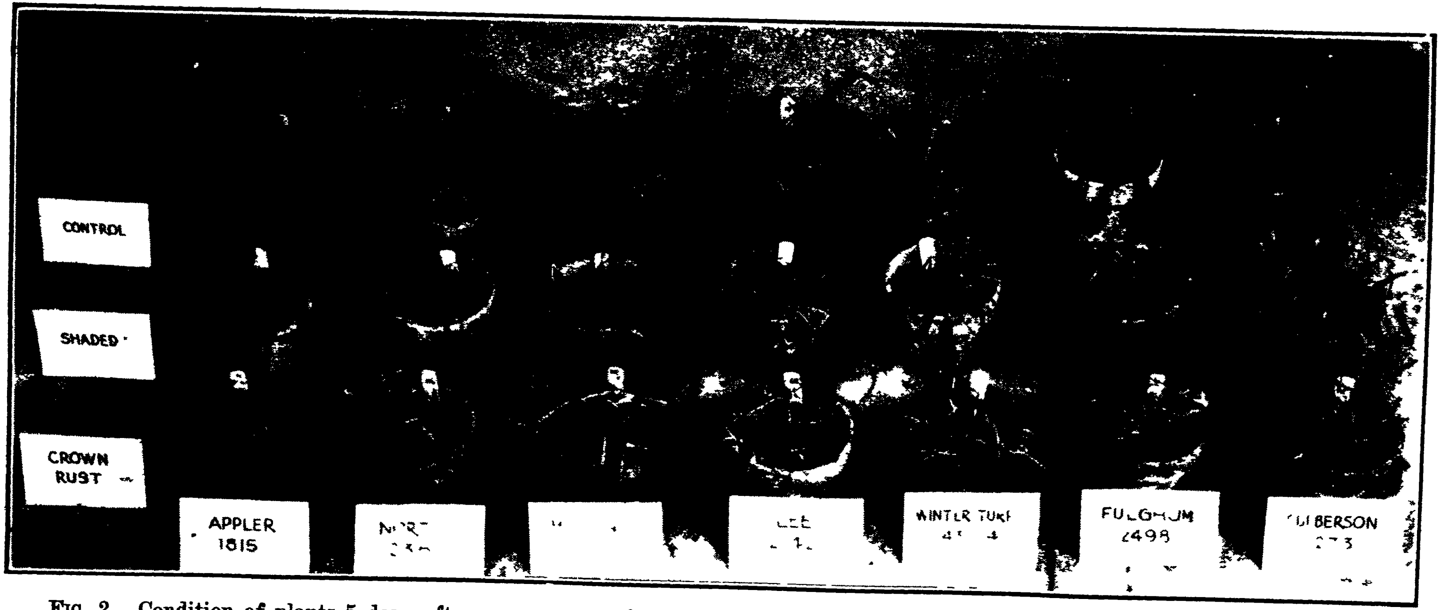

FIG. 2. Condition of plants 5 days after an exposure of 24 hours at 20° F. *Control:* Rust-free plants. *Shaded:* Shaded 10 days with one thickness of gauze. *Crown rust:* Infected with 40 per cent severity of crown rust appearing as chlorotic flecks only. (Experiment 8.)

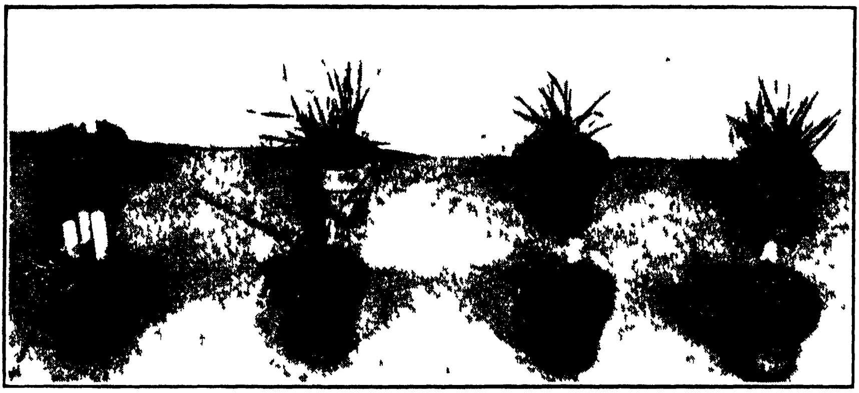

FIG. 3. Survival 30 days after an exposure of 24 hours at 20° F. *Left to right*. Tennessee Fulghum (winter type) selections 1896, 1962, 1906, and 1884. *Upper row* Rust free plants. *Lower row* · Infected with 80 per cent severity of crown rust. (Experiment 10.)

with an exposure of 24 hours at 20° F. Also, the effect of a given infection was less with a short exposure to cold than with a longer exposure. The leaf tissue directly infected with rust usually was entirely killed by any of the freezing exposures. With the more cold resistant varieties the injury often would stop abruptly where the infection ceased, while in the less hardy varieties the remainder of the plant would be killed.

In 13 lots involving plants infected with crown rust in amounts ranging from 20 to 80 per cent with an average infection of 45 per cent, the average decrease in hardiness was 50 per cent; while in 7 lots involving plants infected with stem rust in amounts ranging from 15 to 85 per cent and aver-

TABLE 3.—*Summarized effect of crown and stem rust infections on resistance of 21 varieties to freezing temperatures*

Average amount of infection	Average hardiness index with exposure at 20° F as compared with controls in the same experiments		
	20 hours	24 hours	48 hours
Crown rust	*Per cent*	*Per cent*	*Per cent*
20 per cent		-13	-22
30 do	-37	-42	-58
40 do		-45	
40[1] do		-48	
50 do		-47	-68
60 do		-62	
80 do		-69	
Stem rust			
15 per cent		- 9	
25 do		-13	
40 do		-26	
45 do		-31	
50 do		-52	
60 do		-66	
85 do		-91	

[1] Infection appearing as chlorotic flecks only.

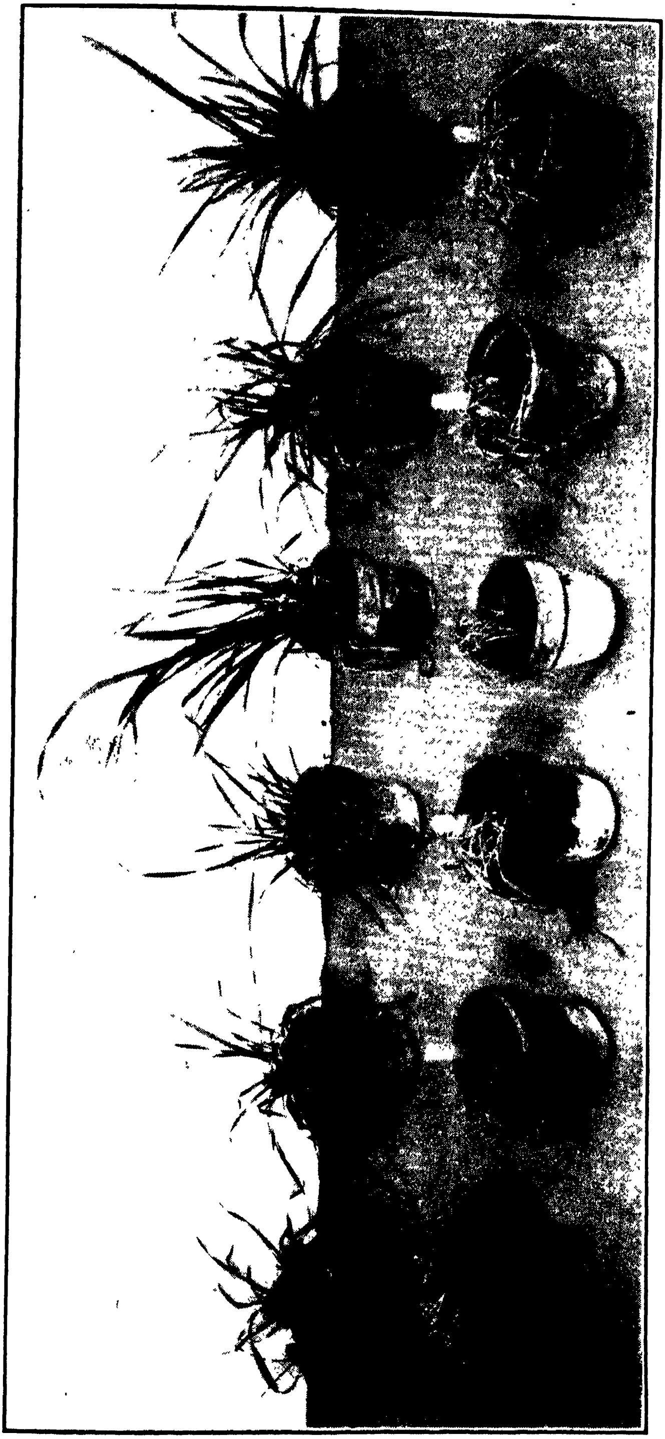

FIG. 4. Survival 30 days after an exposure of 24 hours at 20° F. *Left to right:* Custis, Lee, Nortex, Fulghum (C. I. 708, 2498, and 2500). *Upper row:* Rust-free plants. *Lower row:* Infected with 85 per cent severity of stem rust. (Experiment 10.)

aging 46 per cent the average decrease in hardiness was 41 per cent. This comparison would indicate that with equal amounts of infection crown rust will lower the cold resistance of oats slightly more than stem rust. Data obtained from tests of hybrid populations infected with approximately equal percentages of crown and stem rust indicate, however, that under like conditions of hardening, etc., stem rust lowered the cold resistance of juvenile plants slightly more than did crown rust.

Plants of two different ages were used in experiment 4 (Table 2) to determine whether the effect of crown rust infection on the cold resistance is dependent on age. Plants in the 4-leaf stage showed a hardiness index 16 per cent below that of similarly hardened plants in the 6-leaf stage when exposed 24 hours at 20° F., and a hardiness index 89 per cent lower when exposed 47 hours at 20° plus 1 hour at 0°. A crown-rust infection of approximately 60 per cent lowered the average hardiness index of the 6-leaf plants 66 per cent. In a set of 4-leaf plants with a like amount of infection and exposure, lost by accident before their hardiness index was ascertained, it was evident that they were injured more than the similarly infected older plants.

Plants watered with 1 per cent of $NaNO_3$ and NaCl in experiment 1 showed only slight injury after 2 weeks, and were then exposed 20 hours at 20° F. The average hardiness index was higher than the indices of the control plants. Stronger solutions injured the plants, finally killing those watered with a 2 per cent or stronger solution. In one experiment the resistance of the different varieties to the salt solutions appeared to be definitely related to their resistance to cold, as determined in other experiments. Repetitions of this experiment, however, gave neither similar nor satisfactory differentiation. No further attempt was made to utilize injury from salt solution as an index of relative winter hardiness.

RELATIVE COLD RESISTANCE OF WINTER OAT VARIETIES AND SELECTIONS

Although the 11 experiments reported in table 2 were conducted primarily to determine the effect of rust infection on the cold resistance of winter oats, data also were obtained as to the relative rank in cold resistance of the varieties and selections in each experiment. Since very little information has been published regarding the relative winter hardiness of certain varieties and selections, and in view of the fact that many of the improved varieties and superior strains of winter oats grown in the United States are included in this study, these data should be of interest to oat breeders who wish to develop hardier winter types.

Standard Varieties

The average hardiness index and rank of the 30 oat varieties included in the experiments conducted during the winter of 1933–34 are shown in table 4. Nine of these lots bore 20 to 60 per cent of crown rust, or an average of 39 per cent. In two lots there were stem-rust infections of 25 and 45 per

TABLE 4.—*Average hardiness index and rank of 30 oat varieties with different treatments for experiments conducted during the winter of 1933–34*

Variety	C.I. No.	Crown rust 9[a]		Stem rust 2[a]		Control 14[a]		All 25[a]	
		Ave. hard. index	Rank	Ave. hard. index	Rank	Ave. hard. index	Rank	Ave. hard. index	Rank
Hairy Culberson	2505	52.2	3	88.0	1	75.0	1	68.0	1
Culberson	273	54.4	2	74.0	6	73.3	2	66.6	2
Coker No. 32–1	3026	56.7	1	82.5	2	67.5	3	64.8	3
Fulghum (winter type)	2499	51.1	4	80.0	3	67.4	4	62.5	4
Fulghum (winter type)	2498	50.0	5	75.0	5	66.1	5	61.0	5
Bicknell	3218	48.3	9	72.5	7	65.6	6	60.0	6
Sporen	2506	49.2	7	72.0	8	64.6	7	59.6	7
Culred	3217	50.0	5	72.5	7	62.6	12	58.8	8
Norton	2910	48.9	8	60.0	14	63.0	10	57.7	9
Norton	2909	49.4	6	55.0	15	63.1	9	57.6	10
Winter Turf	3296	45.6	11	65.0	12	63.6	8	57.2	11
Winter Turf	3295	43.7	12	60.0	14	62.8	11	55.7	12
Fulghum (winter type)	2500	43.3	13	72.5	7	61.1	13	55.6	13
Custis	2041	45.7	10	60.0	14	58.6	15	54.0	14
Ferguson No. 71	844	37.9	16	76.0	4	57.4	16	51.9	15
Lee	2042	33.9	17	69.5	9	59.3	14	51.0	16
Tech	947	41.8	14	66.0	11	52.1	18	49.5	17
Burt selection	2684	33.9	17	69.0	10	53.2	17	47.5	18
Appler	1815	41.1	15	65.0	12	44.6	20	45.0	19
Fulghum	708	31.7	18	28.0	19	46.3	19	39.6	20
Nortex	2382	29.2	20	61.5	13	39.8	21	37.8	21
Hastings	2462	31.7	18	55.0	15	39.4	22	37.8	21
Ferguson No. 922	2150	30.2	19	52.5	16	36.6	23	35.6	22
Bond	2733	23.9	21	37.5	17	17.5	25	21.4	23
Alber	2766	15.9	22	30.0	18	19.6	24	19.1	24
Victoria	2401	8.9	23	0.0	22	12.8	26	10.4	25
Markton	2053	6.1	24	5.0	20	12.8	26	9.8	26
Iogold	2329	1.1	25	4.0	21	10.6	27	6.6	27
Avena brevis	1783	1.1	25	0.0	22	2.4	28	1.7	28
Glabrota	2630	1.1	25	0.0	22	2.1	29	1.6	29

[a] Number of lots from which averages and rank were calculated.

cent, or an average of 35 per cent. The remaining 14 lots of plants were rust-free. The varieties are listed in the order of their rank in cold resistance, based upon the average hardiness index for all laboratory tests. These averages probably are more nearly representative of field results than those from the 14 lots of rust-free plants because autumnal infections of both rusts do occur, although possibly not in the relative frequency and severity used in these experiments.

Although infection with either rust greatly weakens a plant with respect to its cold resistance or ability to become hardened against cold, the major differences in rank apparently brought about by rust infection probably resulted from differences in percentage of infection attributable to variation in amount and distribution of the inoculum and to experimental error. There was some tendency for certain varieties and selections to show relatively heavy or light infections, a tendency usually, although not always, reflected in effect of rust infection on the rank of that particular sort. In general, the amount of infection obtained under greenhouse conditions seemed to be determined by the amounts of inoculum; while, under field conditions, the amount of infection may be influenced also by the apparent functional or morphological resistance of the variety. Type of infection, on the other hand, apparently is determined both in field and greenhouse by physiologic resistance of the host. Type of infection appeared to be unimportant in affecting the relative rank; that is, a 40 per cent infection of crown rust of type 0–1 appeared to have about the same effect on the cold resistance of Victoria as a 40 per cent of type 3–4 had on Iogold.

Bond, Victoria, and Markton are spring varieties, but apparently are more resistant to cold than common spring varieties, such as Iogold. It is possible that spring varieties such as these may carry factors for cold resistance that may be combined by hybridization with those from winter types to develop selections more winter hardy than the winter type parent. *Avena brevis* (C. I. 1783) and Glabrota (*A. strigosa glabrescens*) were the most susceptible to injury from cold, apparently having very little, if any, ability to become hardened.

Tennessee Selections Compared with Standard Varieties

The average hardiness index and rank of the 40 varieties and selections included in the freezing experiments conducted during the winter of 1934–35 are presented in table 5. The behavior of the 19 selections from the Tennessee agricultural experiment station, mostly from Fulghum (winter type) (C. I. 2499), is outstanding both in the control lots and in all 18 lots combined. Four of the Tennessee selections (C. I. 3175, 3174, 3168, and 3172) were superior in cold resistance to any of the 21 varieties, while only Culred ranked ahead of Tennessee selection 1922, and, with the exception of Culred and Hairy Culberson, 8 additional Tennessee selections were superior to the remaining 19 varieties. All but 4 of the Tennessee selections appeared higher in cold resistance than the parent variety, Fulghum (winter type) (C. I. 2499), the history of which has been recorded by Stanton (14). Tennessee selection 090 (C. I. 3175) was included in the uniform winter-hardiness experiments, reported by Coffman (1) for the winters 1934–35 and 1935–36. During these two winters this selection showed an average winter survival of 77.9 and 62.7 per cent, respectively, as compared with 72.5 and 52.4 per cent for Winter Turf (C. I. 3296). These data, obtained under field conditions, support the ranking given this selection in this and previous reports (7, 9), which were based upon artificial freezing tests.

Average Results

The average hardiness index and rank of the 21 varieties included in all experiments during both winters and involving rust-infected, shaded, and rust-free plants are presented in table 6. The varieties are arranged in order of their weighted average percentage winter survival under field con-

TABLE 5.—*Average hardiness index and rank of 19 Tennessee selections, mostly from Fulghum (winter type) C.I. 2499 and 21 standard varieties with different treatments for experiments conducted during the winter of 1934–35*

Selection or variety	Tenn. sel. No.	C.I. No.	Crown rust 4[a]		Stem rust 5[a]		Shaded 3[a]		Control 6[a]		All 18[a]	
			Ave. hard. index	Rank	Ave. hard. index	Rank	Ave. hard. index	Rank	Ave. hard. index	Rank	Ave. hard. index	Rank
Tennessee selection	090	3175	63.8	1	55.0	12	80.0	1	95.8	2	74.7	1
Tennessee selection	1962	3174	56.2	3	72.0	1	51.7	3	97.5	1	73.6	2
Fulwin	1945[b]	3168	50.0	5	69.0	2	41.7	8	95.8	2	69.2	3
Tennessee selection	1918	3172	47.5	7	67.0	3	46.7	5	93.3	3	68.1	4
Culred		3217	59.0	2	59.0	7	41.7	8	92.5	4	67.3	5
Tennessee selection	1922	3171	46.2	8	60.4	6	53.3	2	91.7	5	66.5	6
Hairy Culberson		2505	50.0	5	57.2	10	46.7	5	90.0	6	64.8	7
Tenex	1884[b]	3169	52.5	4	62.0	5	43.3	7	85.8	10	64.7	8
Tennessee selection	11053	3173	46.2	8	57.2	10	41.7	8	93.3	3	64.2	9
Tennessee selection	1973		50.0	5	50.4	15	50.0	4	90.0	6	63.4	10
Tennessee selection	1936		46.2	8	63.4	4	36.7	11	87.5	8	63.2	11
Tennessee selection	1967		42.8	9	58.0	9	46.7	5	86.7	9	62.3	12
Tennessee selection	1906		31.2	18	54.0	13	50.0	4	91.7	5	60.8	13
Tennessee selection	1938		41.2	10	58.4	8	36.7	11	85.8	10	60.1	14
Tennessee selection	1879		48.8	6	54.0	11	40.0	9	80.8	14	60.0	15
Culberson		273	41.2	10	43.6	22	40.0	9	88.8	7	57.6	16
Tennessee selection	0114		35.0	15	48.8	16	38.3	10	83.3	12	55.4	17
Winter Turf		3296	35.0	15	46.0	19	38.3	10	84.2	11	55.0	18

[a] Number of lots from which averages and rank were calculated.

[b] Selections 1945 and 1884 were named Fulwin and Tenex, respectively, by the Tennessee Agricultural Experiment Station. Letter from N. I. Hancock to T. R. Stanton, dated Jan. 19, 1939.

TABLE 5.—(*Continued*)

Selection or variety	Tenn. sel. No.	C.I. No.	4[a] Crown rust		Stem rust 5[a]		Shaded 3[a]		Control 6[a]		All 18[a]	
			Ave. hard. index	Rank	Ave. hard. index	Rank	Ave. hard. index	Rank	Ave. hard. index	Rank	Ave. hard. index	Rank
Bicknell		3218	36.2	14	42.0	24	41.7	8	79.2	15	53.1	19
Coker No. 32-1		3026	38.8	12	45.2	30	35.0	12	75.8	19	52.3	20
Winter Turf		3295	40.0	11	48.0	17	23.3	18	78.3	16	52.2	21
Tennessee selection	1921	......	25.0	21	51.0	14	26.7	16	82.5	13	51.7	22
Fulghum (winter type)		2499	32.5	17	45.0	21	26.7	16	82.5	13	51.7	22
Tennessee selection	0113	......	33.8	16	47.0	18	30.0	14	76.7	18	51.1	23
Tennessee selection	095	......	36.5	13	40.0	25	40.0	9	75.0	20	50.9	24
Tennessee selection	092	3170	40.0	11	36.0	29	45.0	6	70.8	23	50.0	25
Sporen		2506	33.8	16	42.8	23	28.3	15	76.8	17	49.7	26
Fulghum (winter type)		2498	32.5	17	39.0	26	33.3	13	73.0	21	47.9	27
Fulghum (winter type)		2500	27.5	19	40.0	25	23.3	18	79.2	15	47.5	28
Tech		947	26.2	20	37.4	28	35.0	12	72.3	22	46.2	29
Tennessee selection	1896	......	27.5	19	38.0	27	25.0	17	73.0	21	45.2	30
Custis		2041	21.2	22	32.0	30	25.0	17	69.2	24	40.7	31
Lee		2042	15.0	24	31.8	31	11.7	19	53.3	27	31.9	32
Ferguson No. 922		2150	17.5	23	24.0	35	1.7	21	57.8	26	30.1	33
Fulghum		708	6.2	28	20.2	36	1.7	21	60.8	25	27.6	34
Appler		1815	13.8	25	26.0	34	3.3	20	48.3	29	26.9	35
Nortex		2382	11.2	26	26.6	33	3.3	20	49.2	28	26.8	36
Hastings		2462	10.0	27	28.0	32	0.0	22	45.8	30	25.3	37
Alber		2766	0.0	29	12.0	37	1.7	21	22.5	31	11.1	38
Iogold		2329	0.0	29	4.6	38	0.0	22	8.0	32	3.9	39

TABLE 6.—*Average hardiness index and rank of 21 oat varieties with different treatments for experiments conducted during winters of 1933–34 and 1934–35, compared with field results obtained by Coffman (1)*

Variety	C.I. No.	Crown rust 13[a]		Stem rust 2[a]		Shaded 3[a]		Control 20[a]		All 43[a]		Field (After Coffman (1))		
		Ave. hard. index	Rank	Ave. hard. index	Rank	Ave. hard. index	Rank	Ave. hard. index	Rank	Ave. hard. index	Rank	Rank	Average per cent survival	Total station years
Hairy Culberson	2505	51.5	2	57.2	2	46.7	1	78.6	1	64.7	1	1	73.5	138
Bicknell	3218	44.6	6	42.0	9	41.7	2	68.6	7	55.5	6	2	72.8	138
Culberson	273	50.4	4	43.6	7	40.0	3	76.9	2	61.4	2	3	72.3	138
Sporen	2506	44.5	7	42.8	8	28.3	7	66.8	9	53.6	8	4	72.2	117
Fulghum (winter type)	2499	45.4	5	45.0	6	26.7	8	70.7	3	56.0	5	5	70.4	138
Fulghum (winter type)	2498	43.1	9	40.0	10	23.3	10	68.9	5	53.5	9	6	69.6	133
Winter Turf	3296	43.8	8	48.0	3	23.3	10	67.1	8	53.9	7	7	68.7	138
Custis	2041	38.2	11	32.0	13	25.0	9	60.5	11	47.0	12	8	68.6	138
Winter Turf	3295	37.2	12	46.0	4	38.3	4	68.7	6	53.3	10	9	67.9	138
Lee	2042	28.1	15	31.8	14	11.7	11	56.1	13	40.6	14	10	67.7	138
Tech	947	37.0	13	37.4	12	35.0	5	56.8	12	46.8	13	11	67.6	138
Coker No. 32–1	3026	51.2	3	45.2	5	35.0	5	68.7	6	57.5	4	12	67.4	55
Culred	3217	52.8	1	59.0	1	41.7	2	70.6	4	61.2	3	13	67.2	138
Appler	1815	32.7	14	26.0	17	3.3	12	43.9	15	35.0	15	14	65.5	138
Nortex	2382	23.7	19	26.6	16	3.3	12	41.2	17	30.8	18	15	63.7	132
Hastings	2462	25.0	17	28.0	15	0.0	14	40.1	18	30.6	19	16	63.5	133
Ferguson No. 2	2150	26.3	16	24.0	18	1.7	13	41.9	16	31.6	17	17	63.0	133
ghum	708	23.8	18	20.2	19	1.7	13	50.2	14	34.2	16	18	61.9	137
Fulghum (winter type)	2500	40.0	10	39.0	11	33.3	6	63.4	10	50.5	11	19	56.9	58
Alber	2766	11.0	20	12.0	20	1.7	13	18.9	19	14.2	20	20	44.4	78
Iogold	2329	0.8	21	4.6	21	0.0	14	9.4	20	5.3	21	.	....	......

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mber of lots from which averages and rank were calculated.

ditions as found by Coffman (1) in cooperative experiments conducted in 13 States over a period of 10 years and representing 138 crop years for most of the varieties. The total deviations of the rankings for the treatments—crown rust, stem rust, shaded, control and all; in comparison with Coffman's field ranking, are 62, 74, 80, 60, and 58, respectively. This would indicate the averages for all 43 lots involving rusted, shaded and rust-free plants were most nearly representative of field conditions, followed in turn by the control and crown rust treatments. Apparently, there was about the same deviation between the rank of different treatments in the laboratory as there was for different years in the field. With additional experiments the results from these laboratory studies might more nearly approach those obtained by Coffman in the field. A number of factors may affect the ranking of the varieties in either series of experiments. The hardiness index as determined in the laboratory is the sole measure of injury resulting from low temperature and is not directly comparable to percentage survival or relative hardiness as determined in the field. While it may afford a fairly accurate measure of the actual cold resistance of the different varieties it does not measure their reaction to alternate freezing and thawing, soil heaving, physiological drought, smothering, etc. Cold resistance probably is usually the most important factor in determining winter hardiness but it is not the only factor involved.

DISCUSSION

The results of these investigations indicate that the effect of autumnal infection of crown or stem rust on the winter survival of oats becomes more pronounced with a heavier infection of rust or a more severe exposure to cold. A light infection of either rust might be a limiting factor for survival under certain winter conditions and a heavy infection probably would result in critical injury from freezing temperatures that would not seriously affect rust-free plants. Early sowing usually increases the chances for autumnal infection; and, with favorable weather conditions, the infection may become heavy and generally distributed. Under such conditions rust may be an important limiting factor in winter survival. Heavy rust infections also tend to eliminate the differences in cold resistance between different varieties. For example, the variety Hairy Culberson, when properly hardened, has considerably more cold resistance than Nortex, but when both are heavily infected with rust during the hardening period, the difference in cold resistance is much less.

Dexter (2, 3) has shown that hardening of wheat plants is favored by conditions that tend toward accumulation or conservation of carbohydrates and other foodstuffs. The writer (11) found that the proportions of sucrose, glucose, and levulose in rusted Markton oat plants were decreased 83.6, 78.7, and 97.4 per cent, respectively, while soluble solids and dextrin showed a decrease of 19.3 and 23.3 per cent, respectively. Rust infection or shading of seedling plants of winter-type oat varieties apparently retards their abilit[illegible] become hardened against injury from freezing temperatures by pre[illegible]ting the accumulation of sugars and other carbohydrates, as occurs when the plants harden in a normal manner, and the retardation is in proportion to the amount and duration of the infection or the degree of shading. It is evident that both of these conditions have a direct effect on photosynthesis. Kokin and Toomarinson (5) and Gretschushnikoff (4) found that photosynthesis (measured by CO_2 assimilation) is increased during the first few days of the incubation period of crown rust but soon falls and remains low, even after the formation of pustules. The decrease in CO_2 assimilation became greater as the amount of infection increased. Kokin and Toomarinson (5) also found that the amount of soluble carbohydrates, proteids, and chlorophyll in the oat leaves decreased with an increase in intensity of infection. Rust infection has a direct effect upon photosynthesis and the conservation and accumulation of carbohydrates and thereby may become an important factor affecting the ability of oat plants to become hardened against injury from freezing temperatures.

It is a well-known fact that cold resistance is only one of the factors determining winter hardiness. By comparing the data obtained from artificial freezing with those from Coffman's (1) uniform winter-hardiness nurseries, it appears that cold resistance and winter hardiness are closely correlated. Artificial freezing offers an expedient method for immediately determining the approximate relative winter hardiness of a group of varieties or for the immediate elimination of tender seedlings from hybrid progenies and should be an aid for more rapid progress in breeding for cold resistance in oats. Both artificial freezing tests and field experiments are subject to considerable variation.

Certain of the Fulghum (winter type) strains selected from Fulghum (winter type) (C. I. 2499) by N. I. Hancock, of the Tennessee Agricultural Experiment Station appear equal to or even slightly superior in hardiness to the standard winter oat varieties. These selections should be of considerable value at least for breeding material. Oat selections with a combined resistance to crown rust and cold, such as those recently reported by Murphy, Stanton, and Stevens (12), should be superior for winter hardiness whenever autumnal infection of crown rust is present. It is a common occurrence, however, that whenever it is cold enough to cause an appreciable amount of winter killing all winter oats will be killed with little differentiation between the more and less hardy varieties. Before marked progress can be made in breeding for greater winter resistance in oats, greater basic resistance to cold must be obtained from some source. The perennial species *Avena pubescens* Huds., *A. hookeri* Scribn., and *A. mortoniana* Scribn. are far superior to cultivated oat varieties in cold resistance. Attempts are being made to cross these species with various oat varieties in the hope of obtaining a source for greater resistance both to cold, and to rusts and smuts of oats.

SUMMARY

Infection with crown or stem rust, or shading, during the hardening period reduced the capacity for juvenile oat plants, grown under greenhouse conditions, to become hardened against injury from freezing. The loss in cold resistance caused by rust infection becomes greater with an increase

either in severity of infection or degree of exposure. Plants in the 4-leaf stage of development were less resistant to cold, whether infected or rust-free, than equally hardened plants in the 6-leaf stage.

The hardiness index of 21 varieties infected with 20 to 80 per cent of crown rust was 13 to 68 per cent lower than similarly treated rust-free plants. Stem rust infections ranging from 15 to 85 per cent lowered the cold resistance of the same group 9 to 91 per cent. Shading with gauze during the hardening period lowered the cold resistance of the varieties. Shading resulting from extraneous rust spores appeared not to be a major factor in lowering cold resistance of infected plants.

Watering oat plants with a 1-per cent solution of $NaNO_3$ or NaCl previous to freezing had a hardening effect somewhat greater than that caused by exposure to a temperature of 38° to 40° F. More concentrated solutions of these salts tended to injure the plants in a manner similar to exposure to freezing temperatures. Repeated experiments, however, indicated that injury from salt solution may not be a reliable index of relative cold resistance.

The average condition value and rank in cold resistance of the varieties and selection included in 43 freezing tests are presented. Hairy Culberson, Culberson, Culred, Coker 32–1, Fulghum (winter type) (C. I. 2499), Bicknell, Winter Turf (C. I. 3296), Sporen, Fulghum (winter type) (C. I. 2498), and Winter Turf (C. I. 3295) ranked highest of the standard winter varieties on the basis of all tests. Certain Tennessee selections from Fulghum (winter type) (C. I. 2499) appeared to be equal to or slightly superior in cold resistance to the most hardy of the standard winter varieties.

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RUST ON JASMINUM GRANDIFLORUM[1]

M. J. THIRUMALACHAR[2]

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Uromyces hobsoni Vize on *Jasminum grandiflorum* L. is the most common rust in India, occurring in both warm and cool regions. It also is reported to occur in India on *J. arborescens* and *J. officinalis,* and on a *Jasminum* sp. in Somaliland, Africa. As far back as 1891, Barclay (2), who named this fungus *Uromyces cunninghamianus,* made a detailed study of its life history. Later, Ajrekar and Parendakar (1) also studied the disease, but their work was concerned mainly with the question whether *U. hobsoni* on *J. grandiflorum* differed from *Uromyces* on *J. malabaricum.* Investigations begun by the writer in 1937 revealed a number of new features of this widespread rust, which are here presented.

SYMPTOMS

The fungus attacks the leaves, stems and flowers, and rarely the fruits, forming orange-colored, swollen, pustulate cushions that ultimately become brownish-black (Fig. 1, A). The affected flower buds are swollen. On the green stems and twigs large oval cankers form. The disease first appears in July-August (during monsoon rains) and continues till March, after which it is dormant until the next outbreak.

MATERIAL AND METHODS

Material, collected in different seasons at Bangalore, Mysore state, was fixed in such fluids as Flemming's weak solution, Allen's fluid, Bouin's fluid, and acetic alcohol. Material for germination and infection studies was collected in large paper bags, and stored in a cool, dry place. Flemming's fluid, Allen's and Bouin's fluid gave good results for fixation of aecia and telia. Acetic alcohol proved very serviceable for fixation of pycnia, although hardening of the host tissue occurred. A 10 per cent aqueous solution of formalin was used for fixation of germinating spores, as recommended by Chamberlain (4). Sections 7.5 to 10 µ thick were cut. Heidenhain's iron-alum haematoxylin, with Orange G in clove oil as a counter stain, gave good results for staining telia and pycnia. For staining aecia, anilin-water aqueous gentian violet gave excellent results, the nuclei being clearly brought out.

SORUS FORMS AND THEIR DEVELOPMENT

Three forms of sori are found in *Uromyces hobsoni,* viz., pycnia, aecia, and telia; uredia being absent. All three forms occur on the hyper-

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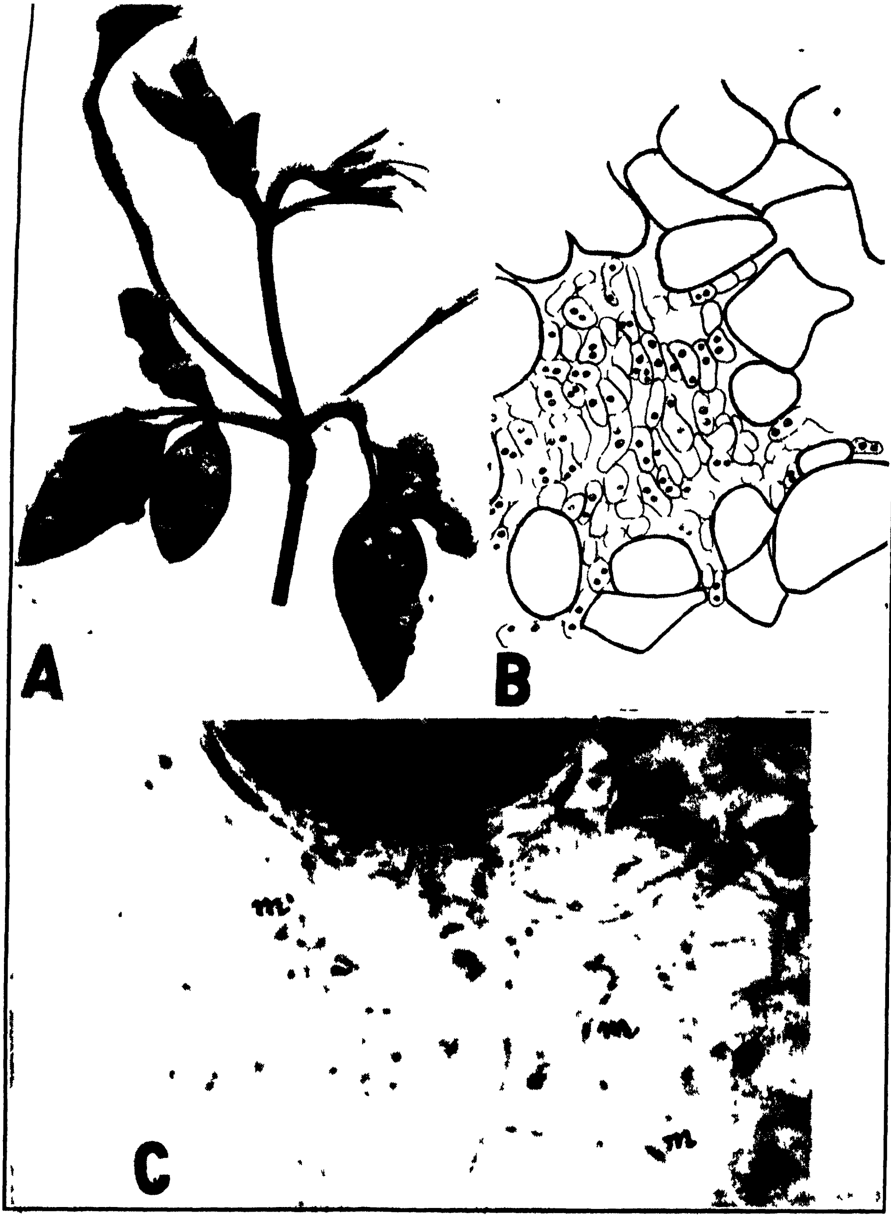

FIG. 1. A. Flowering shoot showing hypertrophy on leaves and flower stalks. × 1. B. Camera lucida drawing showing the early stages of aecial formation. Note the binucleate plectenchyma cells. × 720. C. Section of a flower bud showing the binucleate mycelium (m) associated with the pycnium, of which only the basal portion is seen. × 450.

trophied area of the leaf or stem. There is no definite succession of sorus forms. During the portion of the life cycle from July to April all 3 sorus forms occur in juxtaposition at different periods (Fig. 2, C). Material containing aecia can be collected throughout the life cycle; that bearing telia, a little while after the outbreak of the disease in July; and that containing pycnia, at various periods. Finally, by April, only a brownish-black mass of telia remains.

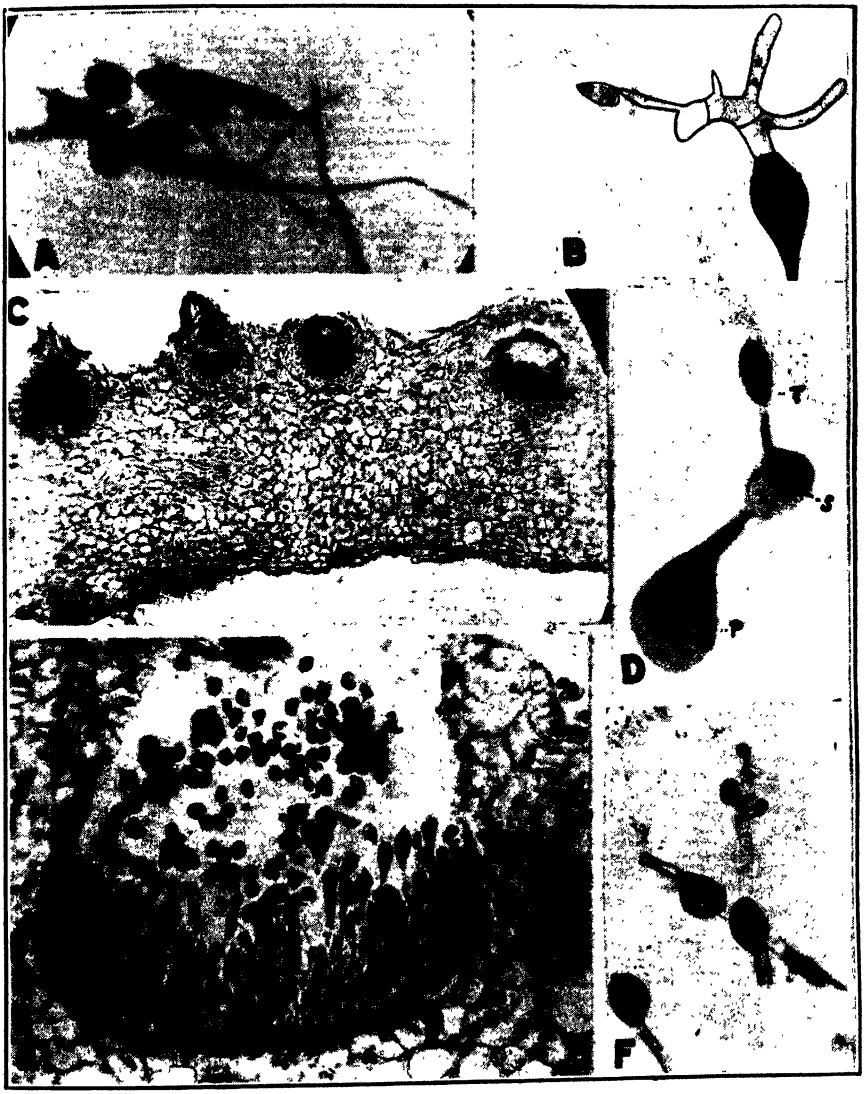

FIG. 2. A. Germination of aeciospores showing the two-celled germ tube, each cell having two nuclei. The whip-like structure enters the stoma during infection. × about 300. B. Germinating teliospore showing sterigmata and binucleate basidiospores. Drawn from a stained preparation. C. Photomicrograph of a transverse section of affected leaf showing in a row three pycnia and an aecium (extreme right). × about 65. D. Germination of basidiospores (P) showing, (S) secondary and (T) tertiary sporidia. E. Photomicrograph showing teliospores developing within an old aecial cup: a, aeciospores. × about 250. F. Germination of teliospore showing three basidiospores photographed from a hanging-drop culture. × about 250.

PYCNIA

Barclay noticed a few pycnia in some of his sections, though he did not find them on intact specimens. Ajrekar and Parendakar observed no pycnia in material collected by them in Bombay. Numerous pycnia were collected by the writer, first in August and, later, in November and January on shoots and flower buds. It was at first difficult to make out the pycnia macroscopically, on account of their close resemblance to unopened aecia. Later, with experience, the writer could detect them by their yellowish-orange color and glistening appearance in the sun. They seem to disappear in a short time.

The pycnia are not restricted to the upper surface of the leaf, as is usual in rusts, but are found on either surface in relation to the initial infection. They are subhypodermal and sunken, with a definite ostiole, possessing numerous projecting hyphae enmeshed in nectar.

The pycnial primordia arise just below the epidermal layer by the massing of hyphae. Generally, in rusts, the pycnia are borne on the gametophytic uninucleate mycelium resulting from germinating sporidia and subsequent host infection. In case of *Uromyces hobsoni,* however, the pycnia-bearing mycelium is always binucleate (Fig. 1, C). The basal cells on which the pycnosporophores arise are also binucleate. This condition is unique, and, in the knowledge of the writer, is the first case recorded among the rusts.

The pycnospores are spherical or ovoid. It is extremely difficult to ascertain the disposition of the nuclei in the minute spores. It was noticed, however, that many of the spores that were shed near the ostioles were binucleate. In some, a nucleus with a large vacuole occupying the major portion of the spore was observed; in others, nuclear reticulation, as reported by Blackman (3) in *Phragmidium violaceum,* was seen. The presence of binucleate mycelium and basal cells in the pycnia is an interesting feature, which may be correlated with the binucleate condition of the pycnospores.

AECIA

The aecia are cupulate, and have a definite pseudoperidium, which ruptures at maturity. The aeciospores are round, bear warty outgrowths, and are formed in chains with sterile intercalary cells between them.

In studying the development of aecia in *Uromyces hobsoni* 2 types of aecia are to be considered:

(1) Those resulting from secondary infection by aeciospores;

(2) Those that develop as a result of primary infection by sporidia. An attempt was made to study the latter type. Material containing pycnia and aecia side by side was selected. The aecia originate deep down in the tissue. Massing of the hyphae in intercellular spaces gives rise to the plectenchyma, the individual cells of which are binucleate (Fig. 1, B). A few cases of cell fusion were observed resulting in the presence of 4 nuclei in the aeciospore initial cell, but this requires confirmation. Regarding the development of ecia resulting from secondary infection by aeciospores, it is interesting to

note the view of Gwynne Vaughan (7), who states that "the cytology of such a rust as *Uromyces hobsoni* would be interesting since it would present the observer with an aecidiosorus which may be different from the typical one because this contains an already binucleate mycelium produced from a binucleate aecidiospore. No cell fusions are necessary in such cases." This question of cell fusion is yet to be investigated. As development proceeds, a chain of aeciospores, alternating with sterile intercalary cells, is formed by repeated division.

OCCURRENCE OF OTHER SPORE FORMS IN PYCNIA

During the first outbreak of the disease, one finds pycnia and aecia distributed promiscuously over the affected surface. After some time, the pycnia entirely disappear, aecia, and later, telia, making their appearance in the same area.

In many of the sections of pycnia, an unusual phenomenon was noticed. From the basal part of the mature pycnial cup, aeciospores and teliospores were seen to develop. In figure 3, A, the basal cells are enlarged and subsequently form teliospores. A more advanced stage is seen in figure 3, C, where the young teliospore has a long stalk and a bulbous head. In figure 3, B, the mature telia can be seen with the remnants of the pycnium at the top.

The first indication of development of aeciospores and teliospores within the pycnium is the enlargement of the basal cells to 2 to 3 times the normal size. Some of these enlarged cells cut off aeciospores, which are binucleate (Fig. 3, D) and, in size, shape, and sculpturing, resemble those developed in normal aecia. This succession of sorus forms is neither exceptional nor abnormal, but has been observed in about 25 different pycnocarps. Obviously, these observations must be restricted to those cases where vestiges of the pycnia are still present. After the complete displacement of the pycnial contents by other sori, it is difficult to say whether the aecial or telial sori originated within the pycnial cup.

The pycniospores, once considered to be noninfectious, degenerate spermatia, have in recent years been shown by Craigie (5) to play an important part in the formation of aecia. In *Uromyces hobsoni,* however, aeciospores and teliospores have been found to develop directly within the pycnia.

Telia

Teliospores are pear-shape, unicellular, with persistent stalks, and are brownish-black. In the mature teliospore, 3 layers are discernible, an exosporium, a continuous thick mesosporium, and a delicate endosporium. The spores are slightly angular and have an apical germ pore.

After the aeciospores are shed, telia begin to develop from within the aecium (Fig. 2, E). Slender hyphae emerge from the base, their tips round off and into these the nuclei migrate. Finally, the spore is cut off by a wall. When the spores are mature, they are opaque owing to the deposition of a

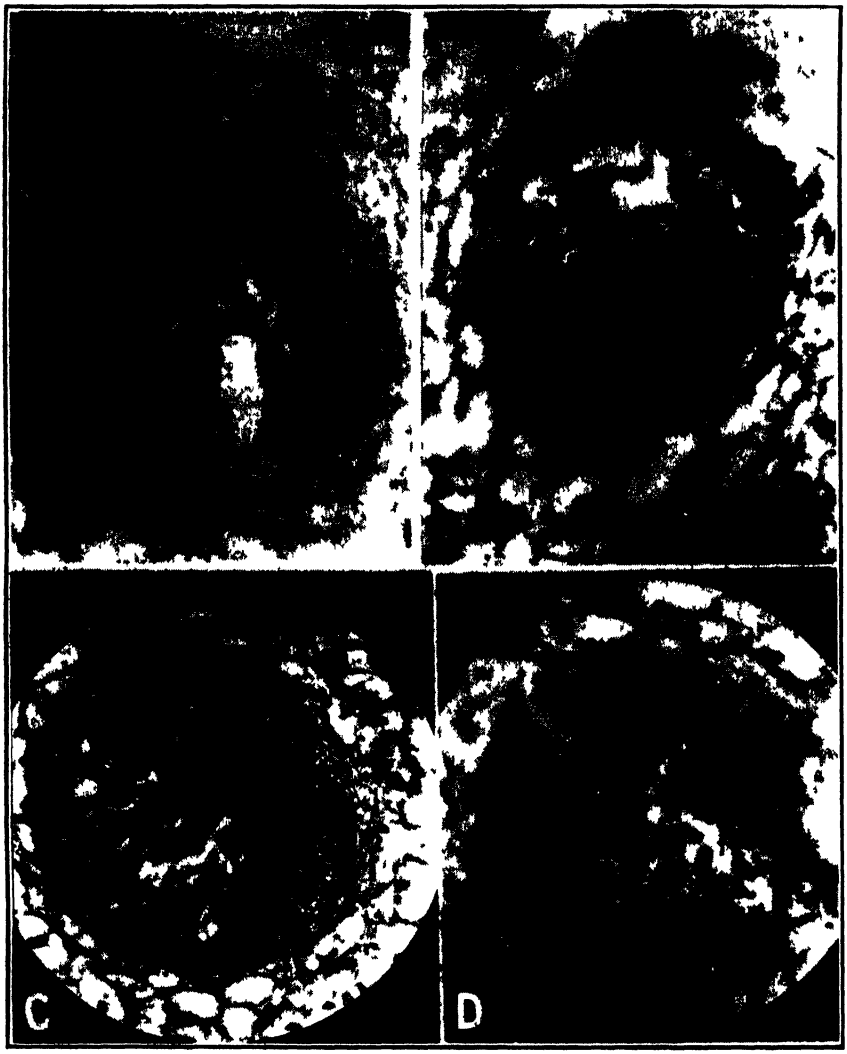

FIG 3. A. Pycnium with enlarged basal cells forming young teliospores (T). Pycnospores (P) are at the top B Advanced stage of telial development within the pycnium. The dark portion above the teliospores indicates a mass of pycnospores. C. Pycnium showing the development, at its base, of teliospores which are stalked, at the place marked (T). D Pycnium showing at its base, early stages of development of aeciospores (a) (P)—pycnospores. A to D. ×270.

yellowish brown substance. Analogous to this type of development is that of *Uromyces alpestris* described by Tranzschel (9), wherein the telia develop within the aecia

GERMINATION OF SPORES

Aeciospore Germination Freshly collected aeciospores germinate readily. Spores collected and stored in paper bags at room temperature lose their germinability after 3 weeks Spores, floated on drops of water, germinated in 24 hours, but submerged spores did not germinate. Nearly 80 per cent germination was secured when spores were dusted on films of water condensed on slides.

After germination, the nuclei migrate into the germ tube, which, by the formation of a septum becomes bicellular. Each cell of the germ tube becomes binucleate. The terminal cell tapers off into a whip-like structure (Fig. 2, A). The lower cell often develops such a structure, usually in advance of the terminal cell. Ajrekar and Parendakar (1) also observed these structures in *Uromyces hobsoni*, and in *U.* sp. on *Jasminum malabaricum,* and considered them to be sterigmata. The presence of one or two "sterigmata" in the germ tube of the aeciospore was interpreted by them to be a differentiating character between *U. hobsoni,* and the species on *J. malabaricum.* The writer's observations show that in *U. hobsoni* itself both the types of development occur. The differentiation of species on this basis, therefore, is not justified.

The term "sterigma" or "sterigmatous branch," as employed by Barclay and by Ajrekar and Parendakar, does not bring out the true significance of these structures. They simulate the whiplike sterigmata of *Endophyllum,* which, however, bear basidiospores at the tip. In *Uromyces hobsoni,* however, these structures appear to be more of the nature of appressoria than sterigmata, as is shown by infection experiments.

Teliospore Germination. For germination of teliospores, a small drop of sterile distilled water was placed on a clean slide and teliospores were added and spread with a glass rod. The slide was next allowed to dry for a few minutes, and then inverted on 2 glass benches in a dish, close to but not touching a water surface. The film of water condensed on the slide afforded ideal conditions for germination, following which the slides were placed in 10 per cent aqueous formalin for 24 hours, stained with Heidenhain's haematoxylin, dehydrated, and mounted in balsam.

Fresh teliospores, collected in July and August, germinated readily and formed sporidia after 3 days, while those collected later in the season germinated after 7 days. With telia collected in Saire (Simla), Barclay obtained a large percenage of germination of the teliospores, but, with material collected at Poona, Ajrekar and Parendakar were hardly successful in germinating them.

The first indication of germination is the extrusion of the stout germ tube, into which the spore contents migrate. When the promycelium attains full growth it becomes 4- to 5-celled. Each cell, but the lowermost, bears a sterigma from the tip of which a basidiospore is rounded off. Variations in the number of cells in the promycelium and in the position of the sterigmata have been observed. Typically, only 3 spores were present (Fig. 2, B and F). All the basidiospores do not develop simultaneously. The sterigmata are branched, in some cases showing 2 nuclei. In other instances each branch develops a basidiospore.

Basidiospores

Basidiospores are oval, flattened on one side, and binucleate. The nucleus in the promycelium migrates into the sterigma and becomes diplodized before entering into the spore.

Binucleate basidiospores have been described in many of the rusts, *e.g.*, *Endophyllum sempervivi* (A. and S.) de Bary, *Puccinia adoxae* Hedw. f., *P. malvacearum* Bert., *Uromyces scillarum* (Grev.) Wint., *Gymnosporangium clavariformae* (Jacq.) DC., *Cronartium ribicola* Dietr., *Phragmidium violaceum*, and *Coleosporium euphrasiae*. Generally, the binucleate condition of the basidiospore arises during the formation of the secondary sporidia, though binucleate primary sporidia also have been observed. Blackman (3), in discussing the nature of the binucleate basidiospore, states that it appears to be entirely without significance, and is due to precocious divisions of the nuclei, in which the usual wall formation is delayed. Poirault and Raciborski (8), working on *Coleosporium euphrasiae*, advance the hypothesis that the sporidia are binucleate and, therefore, to start with, the mycelium is binucleate. Blackman (3), in reviewing their work, concluded that "there is no evidence that the sporidium of any form ever gives rise to anything but a mycelium with a single nucleus, and the condition with paired nuclei always starts in the aecidium when that structure is present." The writer's observations, however, indicate that in *Uromyces hobsoni* this is not the case, for, as has already been pointed out, its gametophytic mycelium is binucleate. This mycelium originates from an infection by a basidiospore. Therefore the binucleate basidiospore leads to the formation of a binucleate pycnial mycelium.

In many cases, the germination of the basidiospores takes place while still attached to the sterigma. Formation of secondary sporidia was often observed.

Secondary sporidia develop even while the spore is still attached to the sterigma. This was also noticed by Barclay. Occasionally even tertiary sporidia have been noticed germinating (Fig. 2, D).

INFECTION EXPERIMENTS

Leafy shoots of *Jasminum grandiflorum*, placed in a moist chamber, were sprayed with water and dusted with aeciospores. After 24 hours the epidermis of the inoculated leaf was peeled off and stained in Lactophenol-cotton blue. It was seen that in the early stages of infection, the long whip-like branch formed from the germ tube entered the stoma. In the later stages, the penetrating germ tube caused distention of the guard cells. Infection through the cuticle was not observed. In inoculated plants the characteristic symptoms, namely, small yellow blisters, were observed after 14 days; 6 days later, aecial pustules appeared.

PATHOLOGICAL ANATOMY

Uromyces hobsoni induces marked anatomical changes in the shoots of *Jasminum grandiflorum*, but does not kill them. Growth stimulation in the infected portions is very marked. Sections through the hypertrophied parts show enlargement of the host cells, ~~and occasional binucleate host cells~~.

The parenchymatous cells become greatly enlarged and the walls become distended, resulting in disappearance of the intercellular spaces. The palisade cells are not differentiated in the affected leaf tissues.

DISCUSSION

The binucleate mycelium associated with aecia and pycnia in *Uromyces hobsoni* is unique. Binucleate mycelium associated with aecia is reported by Moreau (7) in *Endophyllum euphorbiae sylvaticae,* whereby plasmogamy at the base of the aecia is obviated. It is possible, as Gwynne Vaughan suggested (6), that in aecia of *U. hobsoni* formed by secondary infection no cell fusions are necessary. Each cell of the 2-cell germ tube of the aeciospore contains 2 nuclei, so that the resulting mycelium is binucleate.

The mycelium associated with pycnia is also binucleate. Definite evidence of this is obtained during the early stages of floral-bud infection, when no other sorus form than pycnia is present. The basal cells of the pycnia also are binucleate. The binucleate condition naturally arises from the basidiospore, which also is binucleate. Blackman's view, that the binucleate condition in the basidiospore is without significance and results from precocious division of the nucleus, with delayed wall formation, seems not to apply to *Uromyces hobsoni.*

The chief interest in this rust relates to the pycnia. If a group of pycnia is kept under observation for a short time, the pycnia are seen replaced by aecia, leaving no trace of pycnia. Just what happens to the pycnia before the advent of aecial cups remains to be investigated.

It is, however, seen that the pycnia play a new rôle in *Uromyces hobsoni* in that telia and aecia are developed within the pycnial cup. While normally the place of telial development in *U. hobsoni* is within the aecial cup, telia also develop within the pycnia; that is, a structure usually considered in rusts, to be of the sporophytic generation, develops directly within a structure, usually referred to the gametophyte generation. The direct development of telia within pycnia probably is related to the binucleate condition in the pycnia.

Uromyces hobsoni is classified by Engler and Prantl under the Uromycopsis group, on the supposition that pycnia and uredia are absent. In the light of present knowledge, a separate sub-group appears necessary for the reception of this species.

DESCRIPTION OF THE RUST

Uromyces hobsoni Vize. (*U. Cunninghamianus* Barc.) on *Jasminum grandiflorum* L.

Pycnia.—Amphigenis; distributed on shoots and flower buds; orange-yellow, glistening when seen in reflected light; sunken in the host tissue; 160 × 148 µ. Pycnospores spherical or ovoid.

Aecia.—Amphigenis, occurring alongside the pycnia and telia; deep yellow; pseudoperidium present. Aeciospores 14 × 24 µ, thin-walled, minutely tuberculate, germ pore indistinct.

Telia.—Develop within the aecial cup replacing the aeciospores; also arise from the basal cells of pycnia. Teliospores, thick-walled with 3 distinct layers, unicellular, 20 × 35 μ, with persistent stalks; germ pore distinct. Basidiospores, ovoid or spherical, binucleate, 10 × 13 μ, often germinating *in situ* and forming secondary and tertiary sporidia.

SUMMARY

Uromyces hobsoni, an autoecious rust on *Jasminum grandiflorum,* causes hypertrophy of leaves, stems, and flowers.

Three sorus forms (aecia, pycnia, and telia) occur side by side on the hypertrophied portions of the host.

Uredia are absent. The development of aecia proceeds almost throughout the season. Telia develop within the old aecial cups from their base.

Pycnia rarely have been reported hitherto. Numerous pycnia have now been examined. In many cases, aecia and telia were seen to develop within the pycnial cup.

On germinating, aeciospores produce a 2-cell germ tube that develops one or two whip-like structures. These structures are not sterigmata, but are of the nature of appresoria.

Teliospores germinate without rest, and form 3 to 4 binucleate basidiospores. Formation of secondary and tertiary sporidia was observed.

Infection experiments with aeciospores are described. The whip-like structure developed on the germ tube enters the stoma. As a result of infection, secondary aecia are formed.

The mycelium associated with pycnia, and the basal cells of the pycnia are binucleate. The binucleate condition is found in the aecial initials and the aecial mycelium, and in the basidiospores.

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STUDIES ON DRY-ROT CANKER OF SUGAR BEETS[1]

E. L. LECLERG

(Accepted for publication April 5, 1939)

INTRODUCTION

During the summers of 1936 and 1938 dry-rot canker (*Rhizoctonia solani* Kühn = *Corticium vagum* B. and C.) of sugar beets was found in a number of fields in Minnesota and Colorado. The symptoms (Fig. 1) were similar to those described by Richards[2] in 1921.

The present investigation was undertaken to study some of the factors affecting the development of the disease and to learn something about the comparative morphology, physiology, and pathogenicity of various isolates. Comparisons were made also between isolates that cause dry-rot canker, those that cause Rhizoctonia crown rot of sugar beets, and those that attack potatoes.

EXPERIMENTAL RESULTS

Diameter of hyphae

Measurements were made of 200 hyphal diameters of 6 dry-rot-canker isolates and one crown-rot isolate of *Rhizoctonia solani*. The isolates were

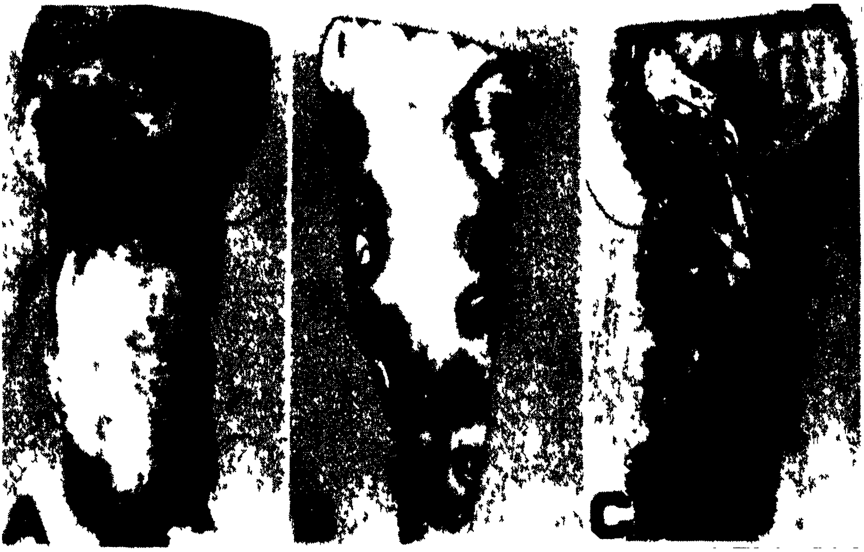

FIG. 1. Symptoms of dry rot canker of sugar beets. A. Typical lesions on roots as a result of natural infection. B and C. Longitudinal sections of naturally infected roots showing various stages of decay.

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[1] The data presented in this paper were obtained in cooperative investigations by the Division of Sugar Plants, Bureau of Plant Industry, United States Department of Agriculture, and the Division of Plant Pathology and Botany of the Minnesota Agricultural Experiment Station. Paper No. 1672 of the Journal Series of the Minnesota Experiment Station.

[2] Richards, B. L. A dryrot canker of sugar beets. Jour. Agr. Res. [U. S.] **22**: 47–52. 1921.

grown on potato-dextrose agar in Petri dishes for 14 days at room temperature before measurements were made. A statistical analysis was made of the data by the analysis-of-variance method.[3]

The average hyphal diameters of the isolates are given in table 1. It appears that there are significant differences between some of the 6 dry-rot-canker isolates but that they are all significantly smaller than the crown-rot isolate.

RELATION OF CULTURE MEDIA TO GROWTH

Six dry-rot-canker, 6 crown-rot, and 6 potato isolates of *Rhizoctonia solani* were grown in duplicate experiments, each consisting of 2 Petri dishes of each isolate, on 3 different artificial media. The results are presented in table 2 and were analyzed by the analysis-of-variance method.

It was found that significant differences exist between isolates within the 3 groups. Thus, on potato-dextrose agar, radial growth of DR-2, SB-13, and P-100 is slower than that of any other isolates of the 3 respective groups. The radial growth of the crown-rot isolates, as a group, is significantly greater than that of the other 2 groups. That of the dry-rot-canker isolates is slightly above the level of significance when compared with the potato group. Greatest radial growth occurred on potato-dextrose agar, followed by low-nitrogen and high-nitrogen. This relationship held for the average of each of the 3 groups of isolates.

TABLE 1.—*Average hyphal diameters of 6 dry-rot-canker isolates and 1 crown-rot isolate of Rhizoctonia solani grown on potato-dextrose agar for 14 days at room temperature*

Isolate[a]	Source of isolate	Average diameter[b] (in microns)
DR-2	Sugar beet, Shakopee, Minnesota	7.4
DR-4	" " " "	7.6
DR-6	" " Longmont, Colorado	7.6
DR-7	" " Greeley, Colorado	8.0
DR-9	" " " "	8.0
DR-11	" " Chaska, Minnesota	7.9
SB-50	" " E. Grand Forks, Minnesota	8.6

[a] DR = dry-rot-canker isolate.
SB = crown-rot isolate.
[b] Difference required for significance = 0.2 micron.

RELATION OF TEMPERATURE TO RADIAL GROWTH IN CULTURE

Six isolates from each of the 3 groups were grown in 2 series on potato-dextrose agar at constant temperatures of 20°, 25°, 30°, and 35° C. The results are recorded in table 3.

The optimum temperature for the dry-rot-canker and crown-rot isolates was 30° C., whereas it was 25° C. for the potato isolates.

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[3] Fisher, R. A. Statistical methods for research workers. Ed. 4, rev. and enl., 307 pp. Oliver and Boyd, Edinburgh and London. 1932.

TABLE 2.—*Influence of 3 different artificial media on radial growth of dry-rot-canker, crown-rot, and potato isolates of Rhizoctonia solani. Results are from two experiments consisting of duplicate cultures of each isolate*

Isolate	Media and radial growth in mm.[a]			Average growth for each group on the 3 media[b]
	Potato-dextrose	Low nitrogen	High nitrogen	
		Dry-rot-canker isolates		
DR–2	26.2	22.8	9.5	22.2
DR–4	34.2	20.2	8.0	
DR–6	38.2	27.8	11.2	
DR–7	32.2	19.5	9.8	
DR–9	34.8	18.0	8.2	
DR–11	35.2	23.0	10.5	
Average[c]	35.1	21.9	9.6	
		Crown-rot isolates		
SB–13	48.2	33.8	12.5	34.6
SB–33	49.8	30.0	15.0	
SB–42	57.0	35.2	19.5	
SB–43	53.0	29.8	14.0	
SB–45	63.2	46.8	19.2	
SB–50	50.2	31.5	14.5	
Average[c]	53.6	34.5	15.8	
		Potato isolates		
P–20	41.0	16.5	6.0	20.6
P–27	45.5	20.8	4.2	
P–85	42.2	23.2	9.5	
P–100	29.8	8.0	1.0	
P–105	43.5	12.2	3.5	
P–116	40.2	16.8	6.0	
Average[c]	40.4	16.2	5.0	

[a] Diff. req. for significance between isolates within groups = 5.9 mm.
[b] " " " " " groups of isolates = 1.4 mm.
[c] " " " " " media within groups = 2.4 mm.

RELATION OF SOIL TEMPERATURE ON THE DEVELOPMENT OF DRY-ROT CANKER

The soil-temperature studies were made in temperature tanks in a greenhouse. The figures given for soil temperatures are the average daily readings obtained from a thermometer thrust to a depth of 2 inches into the soil near the center of the can. Some deviations in temperature, because of surface effects, are to be expected, although the readings from which the averages were obtained seldom varied more than 1° from the mean. Non-wounded steckling sugar beets were planted in artificially inoculated steamed soil in 6-inch pots, and every effort was made to maintain uniform moisture conditions throughout the experiment. Two experiments were made during the winter of 1937.

From the data in table 4 it appears that the dry-rot-canker pathogen is most active at 30° to 35° C. Typical lesions were produced on all inoculated roots at all temperatures, whereas none occurred on the controls. The extent of injury decreased appreciably as the temperature decreased below the optimum. At 15° to 17° C., the rate of decay was exceedingly slow, and the lesions were small and shallow. From the control plants (non-inoculated) it appears that leaf growth is most abundant at 25° C. Marked reduction occurred at 30° and 35° C. Thus, the plants are in an unfavorable environment at the higher temperatures, but these same conditions are favorable for the development of the pathogen.

RELATION OF SOIL MOISTURE TO THE DEVELOPMENT OF DRY-ROT CANKER

Non-wounded steckling sugar beets were grown in artificially inoculated steamed soil in 6-inch pots and received average daily amounts of water, as indicated in table 5. Two experiments were made in the winter of 1937.

From table 5, it is apparent that dry-rot canker of sugar beets is favored by relatively low soil moisture, the extent and amount of decay being most severe under these conditions in both experiments. At high soil moisture, the soil being kept very wet, the lesions were shallow, relatively small, and only a slight amount of decay occurred.

PATHOGENICITY STUDIES

The ability of 6 isolates of the dry-rot-canker group, 6 of the crown-rot group, and 6 of the potato group to cause damping-off of sweet corn, peas, sugar beets, and cabbage was tested in the greenhouse (temperature range 18°–20° C.). Inoculum, consisting of the isolates growing on sterilized oats and wheat grains, was mixed with steamed soil and was allowed to stand about a week before the seed was planted. Sterilized oats-wheat mixture, incorporated into steamed soil, was used for the controls. The experiment comprised 5 replications arranged as randomized blocks within each host.

TABLE 3.—*Average radial growth of 6 dry-rot-canker, 6 crown-rot, and 6 potato isolates of Rhizoctonia solani, as groups, on potato-dextrose agar at four different temperatures*

Group of isolates	Temperature (°C.) and average radial growth (mm.)			
	20°	25°	30°	35°
Dry-rot-canker	20.2	42.0	47.9	9.3
Crown-rot	36.2	62.8	65.7	7.9
Potato	39.8	52.2	32.4	0.5

TABLE 4.—*Influence of soil temperature on the development of the dry-rot canker of sugar beets*

Date of experiment					
February 17, 1937			March 3, 1937		
Temperature (°C.)	Lesions on roots[a]		Temperature (°C.)	Lesions on roots[a]	
	Size	Extent		Size	Extent
15	small	shallow, few	17	small	shallow
20	large	moderately deep	20	moderate	moderately deep
25	do	deep	25	large	do
30	do	very deep, coalescing	30	very large	very deep, coalescing
35	do	do	35	do	do

[a] All plants in noninfested soil remained disease-free.

TABLE 5.—*Influence of soil moisture on the development of dry-rot canker of sugar beets*

Relative soil moisture	Daily amount per root	Lesions on roots[a]		Amount of decay
		Size	Extent	
Experiment 1				
Low	44 cc.	large	deep coalescing	severe
Moderate	75 cc.	do	moderately deep, some	
Experiment 2				
Low	35 cc.	large	very deep, coalescing	very severe
Moderate	95 cc.	moderate	deep, some coalescing	moderate
High	195 cc.	very small	very shallow, no coalescing	very slight

[a] All plants in noninfested soil remained disease free.

TABLE 6.—*Percentage reduction in stand of sweet corn, peas, sugar beets, and cabbage grown in steamed soil infested with dry-rot-canker, sugar beet crown-rot, and potato isolates of Rhizoctonia solani: greenhouse test, temperatures 18°–20° C.*

Isolate	Hosts and percentage reduction in stand[a]			
	Sweet corn	Peas	Sugar beets	Cabbage
Dry rot canker isolates				
DR–2	0.0	22.9	8.1	1.1
DR–4	23.3	51.4	19.1	8.1
DR–6	26.7	28.6	4.4	15.2
DR–7	3.3	25.7	16.9	18.2
DR–9	45.0	70.0	36.0	24.2
DR–11	30.0	15.7	0.0	5.1
Average	21.4	35.7	14.1	12.0
Crown rot isolates				
SB–13	10.0	94.3	42.6	38.4
SB–33	13.3	78.6	25.7	11.1
SB–42	5.0	57.1	79.4	98.0
SB–43	20.0	44.3	25.7	13.1
SB–45	48.3	100.0	100.0	100.0
SB–50	35.0	88.6	80.9	69.7
Average	21.9	77.1	59.0	55.0
Potato isolates				
P–20	1.7	10.0	34.6	34.3
P–27	8.3	14.3	0.0	17.2
P–85	6.7	10.0	0.0	13.1
P–100	0.0	22.4	0.0	0.0
P–105	10.0	12.9	15.4	10.1
P–116	0.0	10.0	6.6	9.1
Average	4.4	13.3	9.4	14.0

[a] Stands in controls taken as 100 per cent in calculation of reduction.

The results given in table 6, which were analyzed by the analysis-of-variance method, indicate that there were marked differences in virulence between isolates in each of the groups on most of the hosts. The dry-rot-canker isolates, as a group, are about equally as virulent as the crown-rot isolates on corn, but are less so on peas, sugar beets, and cabbage. The potato isolates as a group are only weakly virulent on all 4 hosts.

Chlorophyll-deficient corn plants were produced in soil infested with all of the dry-rot-canker isolates, whereas none was present in the controls. The lack of green color was generally localized at the base of the leaves if the entire leaf was not affected. Root systems of affected plants were usually less extensive than those of non-affected plants. The production of albino plants was greatest with isolates DR–4, DR–6, DR–7, and DR–11.

In another experiment, conducted in the greenhouse under similar conditions, 4 dry-rot-canker and 4 crown-rot isolates were tested as to ability to cause preemergence damping-off of beans, peas, sugar beets, and cabbage. The results are given in table 7. For most of the hosts there were marked differences in pathogenicity between isolates of the two groups. All of the crown-rot isolates were strongly virulent to peas, each causing 100 per cent reduction in stand. The crown-rot isolates as a group were more virulent on all 4 hosts than were the dry-rot-canker isolates. This is in agreement with the experiment just discussed.

PATHOGENICITY OF DRY-ROT-CANKER ISOLATE OBTAINED FROM POTATOES

In studies dealing with the effect of soil moisture on the infection of sugar beets with different potato isolates,[4] it was found that one of the isolates (P.162) produced lesions identical with those produced by dry-rot-canker isolates from sugar beets (Fig. 2). Furthermore, low soil moisture favored the development of disease by this isolate, which agrees with the results reported elsewhere in this paper.

Studies of the effect of temperature on radial growth on artificial media of some potato-sprout isolates[4] indicated the optimum to be 30° C. for this same isolate (P.162). As shown above, this was the optimum for 6 dry-rot-canker isolates from sugar beets.

As far as known, this is the first time a potato isolate has been found that caused dry-rot canker of sugar beets.

COMPARISON OF SYMPTOMS PRODUCED BY DRY-ROT-CANKER AND CROWN-ROT ISOLATE ON SUGAR-BEET ROOTS

Since dry-rot-canker isolates were found to differ from crown-rot isolates of sugar beets in morphology, physiology, and pathogenicity on seedlings of various crops, it was thought advisable to conduct an experiment, that would admit a comparison of symptoms caused by a representative isolate of each group on roots of sugar beets.

Inoculum of each isolate (DR–9 and SB–50) growing on sterilized oats-wheat mixture was mixed with steamed soil and allowed to stand 10 days. Sugar-beet stecklings were then planted in the soil in 6-inch pots. The controls consisted of steamed soil with sterilized oats-wheat mixture.

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[4] Unpublished data.

TABLE 7.—*Percentage reduction in stand of beans, peas, sugar beets, and cabbage grown in steamed soil infested with dry-rot-canker and crown-rot isolates of Rhizoctonia solani: greenhouse test, temperatures 18°–20° C.*

Isolate	Host and percentage reduction in stand[a]			
	Beans	Peas	Sugar beets	Cabbage
	Dry-rot-canker isolates			
DR–2	69.1	83.6	16.9	25.3
DR–4	66.2	77.6	4.5	47.0
DR–6	16.2	77.6	19.3	27.7
DR–11	3.0	55.2	0.0	36.2
Average	38.4	73.5	10.2	34.0
	Crown-rot isolates			
SB–13	100.0	100.0	96.8	100.0
SB–33	100.0	100.0	84.0	65.1
SB–42	16.2	100.0	96.2	100.0
SB–45	97.1	100.0	99.5	100.0
Average	78.3	100.0	94.1	91.3

[a] Stands in controls taken as 100 per cent in calculation of reduction.

Very few leaves were produced on roots in the soil inoculated with the crown-rot isolate. The leaves wilted about 10 days after planting and the roots showed typical crown-rot when dug. On the other hand, wilting did not become apparent in the plants growing in the soil infested with the dry-rot-canker lesions (Fig. 2). The plants were slightly stunted when compared with the controls.

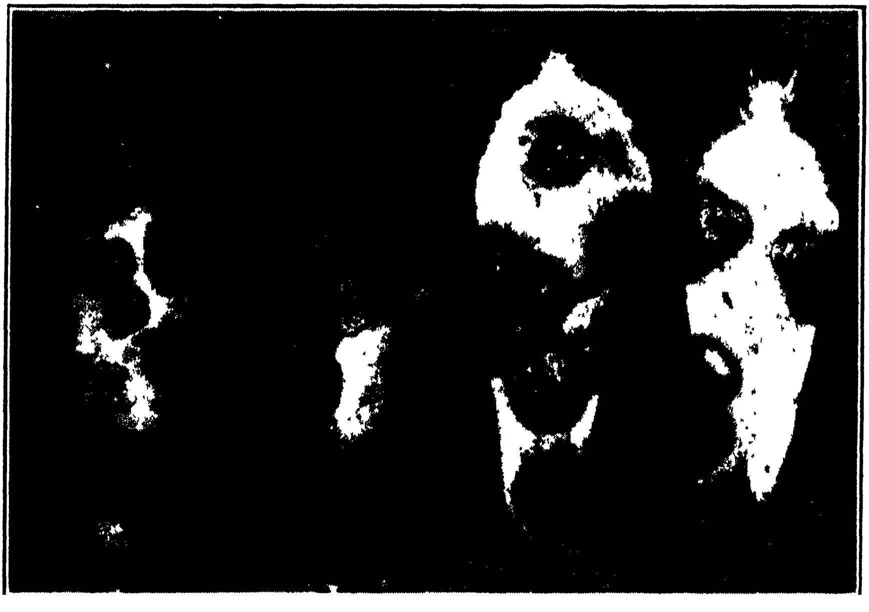

Fig. 2. Infection of sugar beets caused by a dry-rot isolate (DR–9) of *Rhizoctonia solani*. External symptoms are shown on first two roots on left. Internal view of these same roots is shown on the right.

The rotting of roots produced by the inoculations with isolate DR–9 was of the same type as occurs in the field (Fig. 1 and 2). The rather localized, deeply penetrating lesions characteristic of dry-rot canker are in sharp contrast with the generalized decay found in sugar-beet crown rot. This difference in symptoms produced in the sugar beet was completely borne out in the comparison inoculations.

DISCUSSION AND SUMMARY

During the summers of 1936 and 1938, dry-rot canker of sugar beets was found in a number of fields in Minnesota and Colorado. The average diameter of hyphae of 6 dry-rot-canker isolates ranged from 7.4 to 8.0 microns. A comparative study was made of dry-rot-canker isolates with those that cause crown rot of sugar beets and those that attack potatoes. Radial growth of the crown-rot group of isolates on artificial media is greater than that of the dry-rot-canker and potato groups. Growth was most rapid for all three groups on potato dextrose agar, less rapid on low-nitrogen agar, and slowest on high-nitrogen agar. The optimum temperature for radial growth of the dry-rot-canker and crown-rot isolates on potato-dextrose agar is 30° C., whereas it is 25° C. for the potato isolates.

The dry-rot-canker pathogen is most active in causing decay of sugar-beet roots at a soil temperature of 30° to 35° C. and is favored by relatively low soil moisture. The dry-rot-canker and crown-rot isolates, as groups, are about equally virulent in causing reduction in stand of corn seedlings and are more virulent than those of the potato group. Greater reduction in stands of peas, sugar beets, and cabbage is caused by crown-rot isolates, as a group, than by the dry-rot-canker and potato groups. The crown-rot isolates as a group are more virulent on beans than was the dry-rot-canker group.

From the evidence obtained it appears that the dry-rot-canker isolates from sugar beets differ in many respects from crown-rot isolates from the same host. These differences, particularly as regards symptoms on sugar beets, are in most cases of such magnitude as to warrant designation of a different species. However, until the perfect stage of the dry-rot-canker pathogen is found, it seems advisable to maintain the species designation as suggested by Richards.[5]

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[5] See footnote 2.

PATHOGENICITY, SYMPTOMS, AND THE CAUSATIVE FUNGI OF THREE APPLE RUSTS COMPARED[1]

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(Accepted for publication April 25, 1939)

INTRODUCTION

Rusts of apple are widely distributed and common diseases throughout the central and eastern portions of the United States. Their economic importance and the lack of complete knowledge of the peculiar life histories of the causative organisms have induced study by many investigators who have reached conflicting results. The most conspicuous diversity of evidence has been with respect to varietal susceptibility of the apple. It is likely that a great deal of this confusion arose from the fact that, prior to 1929, but one species of *Gymnosporangium* was assumed to be the cause of rust on apples in the eastern United States; whereas it is now known that at least 3 species are involved, namely, *G. juniperi-virginianae* Schw., causing the disease here called "apple rust"; *G. clavipes* Cke. and Pk., commonly causing "quince rust"; and *G. globosum* Farl., the usual cause of "hawthorn rust." *G. juniperi-virginianae* occurs on both leaves and fruits of the apple, *G. clavipes* occurs on the fruits but at most is only indistinctly evident on the leaves, and *G. globosum* occurs on the leaves but not on the fruit.

In Indiana, in 1929 (8), apples were observed affected by what was then generally regarded as the common apple rust with symptoms atypical for this disease; and it was found that these apples actually were infected by the quince rust organism, whose specific effects upon the apple were not then generally recognized and distinguished. Surveys during the same year showed that still a third species, the hawthorn-rust organism, was also very prevalent on certain varieties of apples, causing symptoms hardly distinguishable from those of apple rust. Since 1931 there has been an unusual opportunity, in connection with the Plant Disease Survey, to continue the study of these rusts and their causative fungi in some of the more important apple-growing regions of the eastern United States.

IDENTITY OF AND DISTINCTION BETWEEN THE CAUSATIVE FUNGI

Kern (6) published a discussion of the morphology and taxonomy of the genus *Gymnosporangium,* including descriptions of species. Arthur (2) added his annotations to the descriptions given by Kern. Taxonomic characters such as size of spores and of peridial cells have been extensively utilized by Thomas and Mills (13).

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[1] This and the following paper are revisions of sections of a thesis submitted to the Graduate Council of The George Washington University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] The writer is indebted to Dr. W. W. Diehl and to Jessie I. Wood for helpful criticism of the manuscript.

Specimens of the 3 fungi collected by the writer in Indiana, New York, Arkansas, Tennessee, and Virginia furnished material for measurements of aeciospores and teliospores, as well as for observations of additional useful diagnostic characters, such as size of peridial cells, spore color, markings, and thickness of walls, number and location of germ pores, and shape of the teliospore pedicel. The characters of the 3 fungi, based upon observations of these specimens, are compared and contrasted in table 1.

TABLE 1.—*Comparison of the characters of the 3 species of Gymnosporangium*

Character	*Gymnosporangium juniperi-virginianae*	*Gymnosporangium globosum*	*Gymnosporangium clavipes*
		Aeciospores	
Color of spore walls	Light chestnut brown	Light chestnut brown	Pale yellow
Size of spore	16–24 x 21–31 chiefly 22 x 27[a]	15–19 x 18–25 chiefly 18 x 21	21–32 x 24–39 chiefly 25 x 31
Width of wall	2–3	2.5–3	3–4.5
Color of spore in mass	Dark brown	Dark brown	Bright orange
Marking of spore	Finely verrucose	Finely verrucose	Coarsely verrucose
Number of pores	8–10 distinct	8–10	6–10 obscure
Shape of aecia	Cylindric at first, then fimbriate and revolute	Cylindric, soon splitting in upper part, margin lacerate or fimbriate	Cylindric, becoming lacerate to base
Color of peridial cells	Gray	Gray	White
Size of peridial cells	90 x 20 ±	63 x 20 ±	65 x 30 ±
Shape in face view	Not usually seen	Broadly lanceolate	Polygonal-oblong
Shape in side view	Long and narrow	Linear-rhomboidal	Rhomboidal
Reaction of peridial cells to water	Much curved when wet	Remain straight when wet	Remain straight when wet
		Teliospores	
Shape of telia	Cylindric-acuminate	Tongue-shaped	Hemispheric
Length of telia	5–30 mm.	6–12 mm.	1–3 mm.
Color of telia	Golden brown	Chestnut brown	Orange brown
Shape of teliospores	Rhombic-oval	Ellipsoid	Ellipsoid
Size of teliospores	15–21 x 42–65	16–21 x 37–48	18–26 x 35–51
Color of wall	Cinnamon brown	Cinnamon brown	Yellowish
Thickness of wall	1	1–2	1–2
Location of pores	Near septum	Near septum	Apical in upper cell, near pedicel in lower
Number of pores	Two in each cell	Two in each cell	One in each cell
Shape of pedicel	Uniform in width	Uniform in width	Carrotiform

[a] Dimensions in microns.

COMPARATIVE SYMPTOMATOLOGY

Figures 1, 2, and 3, and a summary table (Table 2), comparing and contrasting the symptoms caused by the 3 species of rust fungi, are presented as aids to diagnosis.

APPLE VARIETAL SUSCEPTIBILITY TO THE RUST FUNGI

Numerous lists recording susceptible and resistant apple varieties have

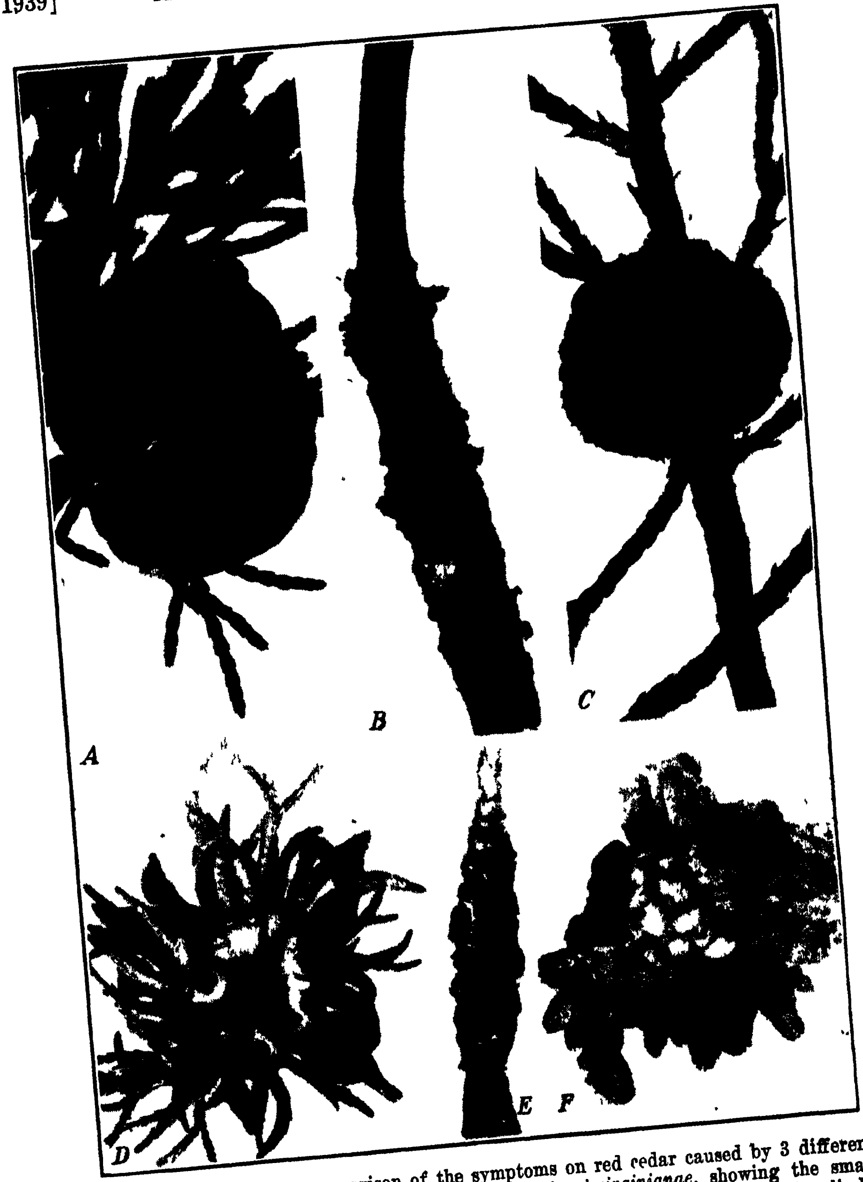

FIG. 1. A to C. A comparison of the symptoms on red cedar caused by 3 different rust fungi. A. Gall of apple rust, due to *G. juniperi-virginianae*, showing the small circular depressions from which telial horns will later protrude. B. Red cedar limbs showing spindle-shape, encircling lesions caused by *G. clavipes* as they appear in the winter condition. The roughened bark is about the only evident symptom at this stage of development, which makes the disease difficult to detect. C. Gall of hawthorn rust, due to *G. globosum*, showing the large, dark-colored, elevated areas, from which spore-horns will later emerge. D to F. The three species of *Gymnosporangium* on red cedar as they appear with teliospore sori gelatinized and expanded. D. *G. juniperi-virginianae* gall, showing cylindrical sporehorns. E. *G. clavipes* on stem showing broad wart-like spore masses. F. *G. globosum* gall, showing large tongue-shape spore masses.

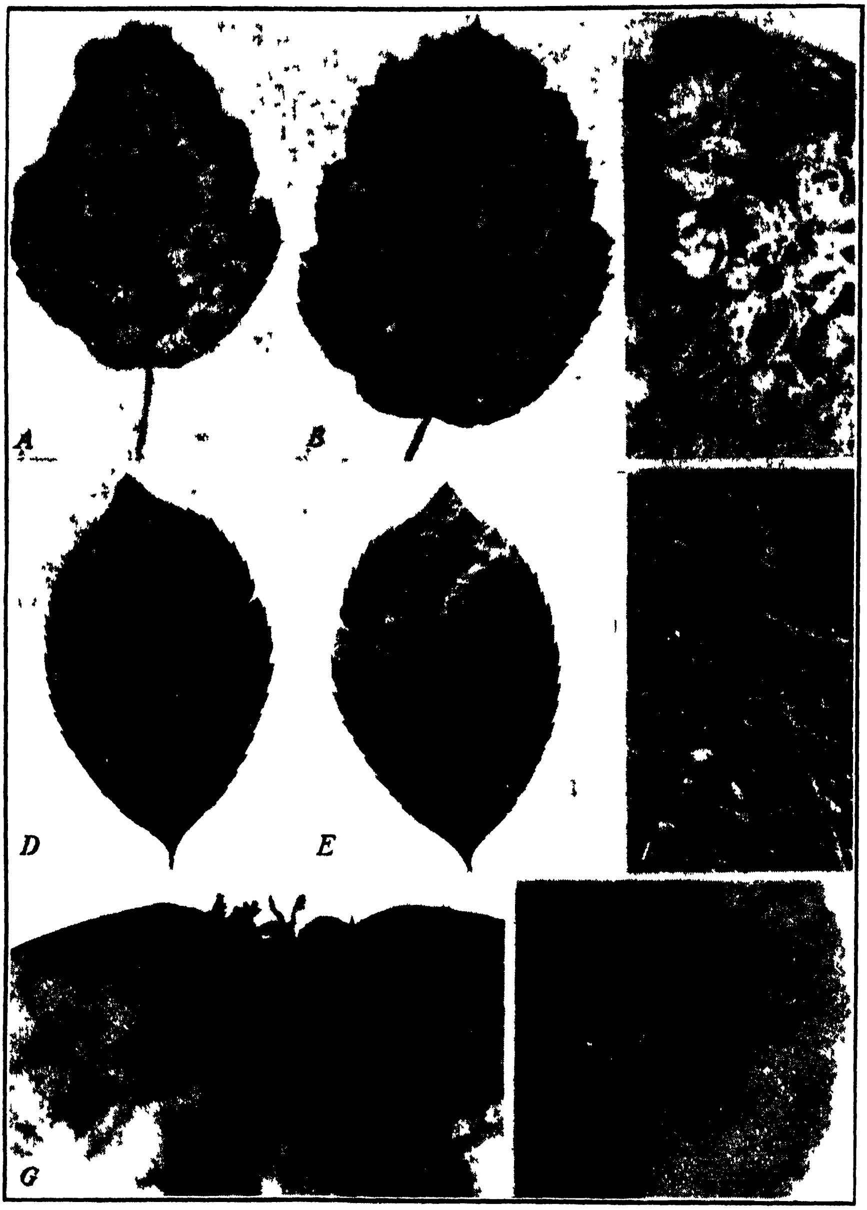

FIG. 2. A to C. Wealthy apple leaves exhibiting unusually conspicuous symptoms caused by *G. juniperi-virginianae*. A. Upper surface with scattered black pycnia in center of large chlorotic lesions. B. Lower surface showing chlorotic areas and a ring of aecia on each lesion. C. Much enlarged section of leaf illustrated in B. Note the shredded, recurved peridia. D to F *G. globosum* on Rome apple leaves. D. Upper surface showing pycnia in centers of small lesions. E. Lower surface, showing from 3–8 aecia aggregated in the center of lesions. F. Much enlarged section of leaf illustrated in E. Note the long tubular, irregularly dehisced peridia. G and H. Calyx end of Rome apple infected with *G. clavipes*. H, external view showing necrosis and aecia; G, section showing killing of internal tissue to a considerable depth.

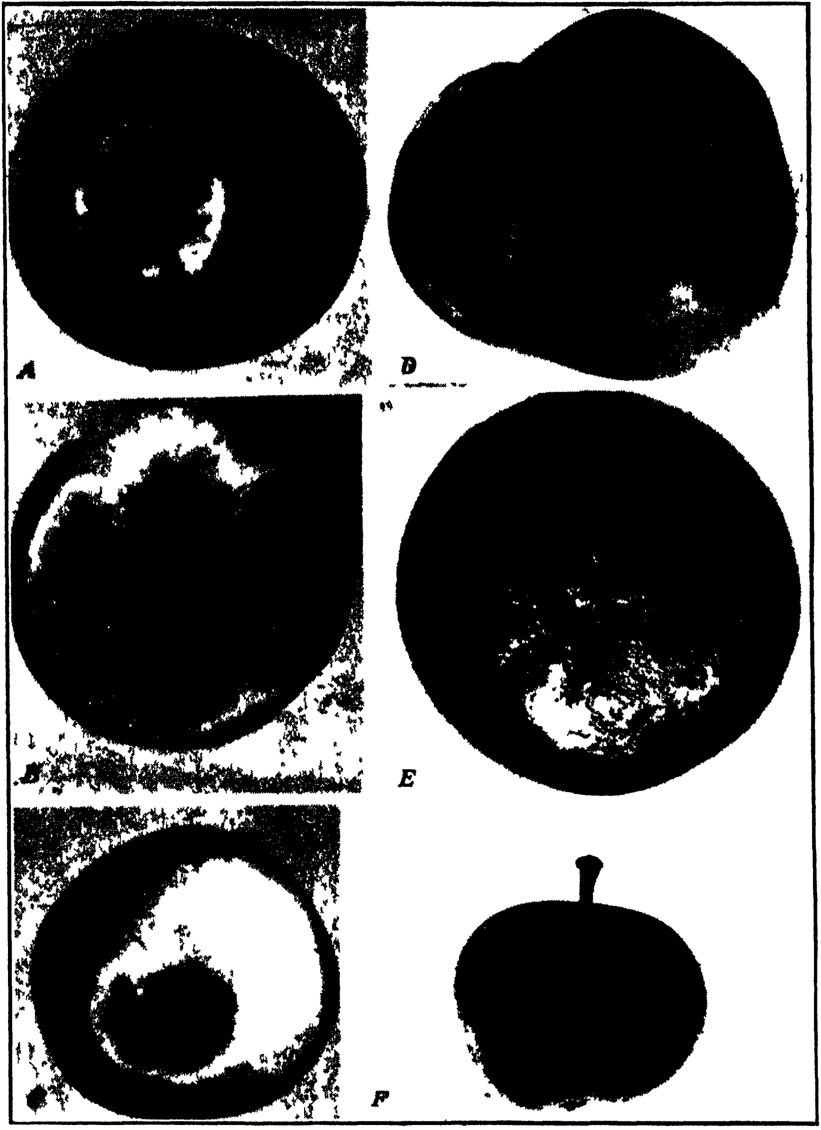

FIG. 3. Apple and quince rust symptoms on fruit compared. A. Rome apples with large orange colored lesions at the calyx end, caused by *G. juniperi-virginianae.* These apples show no distortion and were without any internal necrosis. The lesions are covered with aecia. B. Rome apples with very large orange colored lesions caused by *G. juniperi-virginianae,* showing unusually severe distortion and abundant aecia. Internal sections revealed no necrosis such as is always observed in apples infected with *G. clavipes.* See Fig. 2, G. C. Rome apples with large orange colored lesions caused by *G. juniperi-virginianae,* without distortion, pycnia, or aecia. Note the halo effect surrounding each lesion. This halo never occurs in the case of the quince rust disease. D and F. Delicious apples showing stunting and malformation caused by *G. clavipes.* (In quince rust lesions typical for this variety the tissues are killed while the fruits are small and, because of the unequal growth, misshapen fruit results.) E. Black Twig apples with water-soaked dark-green lesions involving a large area of the fruit surface, typical of the symptoms caused by *G. clavipes* on the Winesap group of apples. A color illustration would be necessary to demonstrate these symptoms adequately.

TABLE 2.—*Summary of comparative symptomatology of the 3 rusts*

Criteria	Apple rust caused by *Gymnosporangium juniperi-virginianae*	Hawthorn rust caused by *G. globosum*	Quince rust caused by *G. clavipes*
On Red Cedar			
Origin of gall	Either stem or leaf	Usually young stem	Young stem
Color of gall	Greenish-brown	Reddish-brown	No color other than bark except when sporulating
Shape of gall	Spherical	Spherical	Cylindrical or spindle-shape
Size of gall	½–8 cm. in diameter	2–5 cm. in diameter	2–60 cm. in length encircling limbs from ¼ to 6″ in diameter
Point of origin of telial horns	Emerge from small pitlike depressions	Emerge from elevated wedge-shape areas	Emerge from roughened bark
Duration of gall	Usually produces spores 1 year only	Usually produces spores from 3 to 5 years	Perennial—may produce spores for 20 consecutive years
Shape of telial horns	Cylindrical	Tongue shape	Short, knob or wart-like
Color of telial horns	Light brown	Brown	Reddish-brown
On Apple Foliage			
First evidence of infection	Small chlorotic spots ⅓ mm. in diameter	Small chlorotic spots ⅓ mm. in diameter	Does not produce evident symptoms other than chlorotic spots, which become indistinct as the leaf develops
Average size of mature lesions	About 12 mm. in diameter	About 4 mm. in diameter	
Color of mature lesions	Yellowish-orange often bordered by red band or chlorotic halo	Light yellowish orange	
Number and location of aecia	5 to 30 usually in a circle or scattered	3 to 8, usually aggregated in center of lesion, or along a vein	
Shape of peridium	Chimney-like at first, then splitting becoming strongly revolute	Longer than *G. juniperi-virginianae*, dehisce irregularly, never becoming revolute	
On Apple Fruit			
Color of lesion	Orange or yellow, frequently surrounded by halo	Does not produce evident symptoms on fruit	Dark green, never yellow
Size of fruit lesion	Average about ¼ to ¾ inches in diameter		Average about ¾ to 1½ inches in diameter
Distortion of fruit	Usually none		Usually very pronounced
Extent of fungus penetration	Superficial, usually not over 1/16 inch		Usually extends to the core
Location of lesion	Usually at calyx end of fruit		Practically 100% at calyx end
Occurrence of aecia	Frequently on susceptible varieties		Rarely observed
Internal necrosis	None		Extends to core

been prepared by various observers from different localities. Tabulation of these records (Table 3) reveals considerable difference in opinion concerning the susceptibility or resistance of certain varieties. The reasons for these differences cannot be definitely detrmined in any individual case, but the following general explanations are suggested: I. Actual differences in the amount of infection induced by variation in environment and types of resistance, reflected in the respective observers' estimates of the degree of resistance or susceptibility. Miller (10) has shown by a mathematical comparative method that the percentage area of apple leaves infected is dependent upon the number of red cedars and their distance from the apples. He also has demonstrated (8) that apple leaves possess at least 2 types of rust resistance. One, inherent in the variety regardless of host maturity; the other, conditioned by the stage of maturity, is recognized in susceptible varieties when mature leaves, which are normally resistant, succumb to infection after wounding. Whether varieties possessing resistance of the latter type would be classified in the orchard as resistant or susceptible might well depend upon the time or place the observations were made. II. The existence of physiologic races within the species. Bliss (3) and, more recently, McNew (7) have demonstrated the presence of races within the species *Gymnosporangium juniperi-virginianae.* Miller (8, 9) has done likewise for *G. clavipes.* III. Gradual adaptation of the fungus to hitherto resistant varieties. Waite (14) hypothesized a change in pathogenicity of *G. juniperi-virginianae.* He states, "Whatever theory or explanation may be advanced, the facts are that this fungus gradually attacked one variety after another with increasing severity. Since localized infections have already begun on the Winesap group and other varieties previously resistant, it is doubtful whether any variety of apple can be counted on as resistant to this disease." The writer has never observed *G. juniperi-virginianae* on the fruit of any of the Winesap group of apples; neither was he able to inoculate them successfully with this fungus. On the other hand, the quince-rust fungus (*G. clavipes*) does infect these varieties throughout most of the apple-growing regions of the eastern half of the United States. Since its effects were for a long time confused with those of the former species, it is natural that infections of a rust reported on the Winesap group should have been ascribed to the apple-rust fungus on the mistaken assumption that its infective powers had in some way changed so as to enable it to attack varieties previously known or considered to be immune. Miller (8), however, has shown that *G. juniperi-virginianae* has a sexual cycle comparable to that described by Craigie (4) for *Puccinia graminis* (Pers.) Eriks.; and it follows that factors for virulence may segregate in the teliospore and so recombine in the aecium as to give rise to a physiologic race capable of causing infection on Winesap or any variety hitherto immune IV. The presence or absence of the different species of the rust fungi (11). The unrecognized presence of the quince and hawthorn rust fungi on apple, in the writer's opinion, is the most important factor contributing to disagreement as to varietal susceptibility, and is, moreover, sufficient in itself to explain the discrepancies.

Quince rust on apple undoubtedly occurred for many years before it was recognized as a distinct disease of importance. In the files of the United States Department of Agriculture are colored illustrations of deformed Winesap apples, collected in 1910 in Virginia, labeled "frost injury," showing external and internal symptoms indistinguishable in appearance from those produced by *Gymnosporangium clavipes* on this variety. John W. Roberts, commenting on the illustrations, states in correspondence, "In 1910 I was stationed in Virginia and know positively that apples were severely injured by frost in that State in that year. The season of 1910 was an extremely early one, apples being in bloom around Waynesboro on April 4. Later in the month when apples had attained a fair size there was a severe frost affecting apples growing in low places. Many of these apples were particularly affected near the calyx, and some were actually frozen so that a dead sheath appeared just underneath the skin." It is, of course, impossible at this date to determine positively the presence or absence of quince-rust infection, as well as frost injury, in Virginia orchards in 1910, but the striking similarity in appearance of the specimens illustrated and quince-rust injury is of interest in either case. According to Gardner (5), in 1924, "On Oldenburg, Delicious, and particularly Gideon, as observed in Orange County [Indiana], many of the fruit lesions were very atypical in that no waxy yellow elevated areas bearing pycnia or aecia were present. In these cases the infection had caused a puckering and internal brown necrosis of the tissues about the calyx end. Sections showed that the affected tissues were rather extensively permeated by the intercellular rust mycelium." His illustrations are typical of infection caused by *G. clavipes,* rather than as he reported it, *G. juniperi-virginianae.* In a letter dated October 18, 1928, to H. E. Thomas, Mr. Hockey of Nova Scotia said that previous to 1924 apple rust was reported from Nova Scotia as caused by *G. juniperi-virginianae.* A very careful survey in subsequent seasons has established that the disease there was caused by *G. germinale* [*clavipes*]. Even as late as 1935 Anderson (1) said, "Deformed apples from western Illinois were received from county advisers and growers during the late spring when the fruit was about one-half inch in diameter. This injury resembled hail damage but those sending in specimens reported no hail. Later, from larger specimens, it was determined that the disease was caused by *G. clavipes.*"

The writer has observed quince rust on apple fruits in amount sufficient to be of commercial importance in New York, Pennsylvania, Maryland, Virginia, West Virginia, North Carolina, Kentucky, Tennessee, Indiana, Illinois, Arkansas, and Missouri.

The hawthorn rust, which can be distinguished only with difficulty from apple rust on the basis of leaf symptoms, even when one is aware of its presence, has surely added to confusion in the literature relative to susceptibility of apple varieties, since it is not known to occur on apple fruit, as does the apple rust. Two early records of its occurrence are given. There are specimens of this disease on cultivated apple collected in Pennsylvania, 1881, now deposited in the Mycological Collections of the U. S. Bureau of Plant Industry. Thaxter (12), in 1891, working in Connecticut, states that perhaps the most common orange rust of apples was caused by *Gymnosporangium globosum*. *G. globosum* on apple foliage has been observed by the writer in Indiana, New York, Arkansas, Georgia, Tennessee, and Virginia.

In an effort to clarify some of the differences concerning apple varietal susceptibility shown in table 3, 9 of these same varieties were inoculated with all 3 of the rust fungi, with results as summarized in table 4. These results were then compared with those of the State investigators (Table 5) by converting their symbols 0, 1, 2, and 3 into those used in table 4, S and R (susceptible and resistant). As can be seen, the findings agree in that most of the varieties may be either susceptible or resistant, depending upon the species of rust with which they were inoculated. For example, if one considers the variety York, it can be seen (Tables 3 and 5) that some workers have reported it as resistant, while others have reported it as sus-

TABLE 3.—*Susceptibility of apple varieties to "rust" (thought to be caused by Gymnosporangium juniperi-virginianae), as reported by workers from various States*

Variety	State																	
	Alabama	Connecticut	Delaware	Indiana	Iowa	Maryland	Massachusetts	Minnesota	Nebraska	New Hampshire	New York	North Carolina	Rhode Island	Pennsylvania	South Carolina	Virginia	West Virginia	Wisconsin
Arkansas Black			0[a]			1						1					0	
Baldwin		1					1				1		1	1			0	
Ben Davis			1		3		1		1				1			3	2	
Black Twig	1		0													1	1	
Bonum			3									3				3		
Fameuse					1	3												1
Fallawater			3									3						
Grimes	2	1	3	1	3	1			1						3	1	1	
Jonathan	3			3	1				3					3		3	3	2
Maiden Blush	1		1			3			1					1			1	
Northern Spy																3		
N. W. Greening		1														1	2	
Red June	3		0						3			3			3			
Red Astrachan	1		0			1	1		1				1					
Rome	3		0	3		3					2	3		3			3	
Shockley	3											3			3			
Stayman			0	2								1		1		1	0	
Wealthy		3		3	3	3	3	3	3	3			3				3	3
Winesap	1			2		1			1		2	1		1		2	0	
Yellow Transparent			0			1	1		1				1				0	
York Imperial	2											1		3		3	3	

[a] The numbers refer to the degree of susceptibility or resistance. 0 = Immune, 1 = Resistant, 2 = Moderately susceptible, 3 = Susceptible.

TABLE 4.—*Susceptibility of fruits and leaves of apple varieties to Gymnosporangium juniperi-virginianae, G. globosum, and G. clavipes, according to results of inoculations*

Variety	Results of inoculations with			
	G. juniperi-virginianae		*G. globosum*	*G. clavipes*
	On fruit	On leaves	On leaves[c]	On fruit[d]
York	S[a]	S	S	R
Wealthy	S	S	S	S
Grimes	S	S	S	R
Rome	S	S	S	R[e]
Ben Davis	S	S	S	R
Jonathan	R[b]	S	S	R
Maiden Blush	R	R	S	R
Stayman	R	R	R	S
Winesap	R	R	R	S

[a] Susceptible.
[b] Resistant.
[c] *G. globosum* does not occur on apple fruit.
[d] *G. clavipes* does not occur on apple leaves.
[e] This variety was susceptible to one physiologic race and resistant to others.

TABLE 5.—*Susceptibility of apple varieties to "rust" recorded under the name Gymnosporangium juniperi-virginianae, according to workers, in various States, compared with the results of inoculations of the same varieties with the 3 species, and suggested explanation for apparent discrepancies in State reports*

Variety	Reported reaction according to States	Reaction based upon inoculations with the three species		Suggested explanation for discrepancies in State reports
York	1[a], 2, 3 = S R	S	R	More than one species involved
Wealthy	3 = S	S		Consistent
Grimes	1, 2, 3 = S R	S	R	More than one species involved
Rome	0, 2, 3 = S R	S	R	do
Ben Davis	1, 2, 3 = S R	S	R	do
Jonathan	1, 2, 3 = S R	S	R	do
Maiden Blush	1, 3 = S R	S	R	do
Stayman	0, 1, 2 = S R	S	R	do
Winesap	0, 1, 2 = S R	S	R	do

[a] S 2 = Moderately susceptible.
3 = Susceptible.
R 0 = Immune.
1 = Resistant.

ceptible. Likewise, the results of the inoculations with the 3 species of rust fungi on York (Table 4) show that this variety is susceptible to *Gymnosporangium juniperi-virginianae* and *G. globosum*, and resistant to *G. clavipes*. Both susceptibility and resistance were shown in the other 8 varieties, with the exception of Wealthy, which was consistently recorded as susceptible by the State pathologists and was also found to be susceptible to the inoculations with all 3 species of fungi.

The inconsistencies in the records are readily understandable if it is assumed, from the evidence presented, that the 3 species of rust fungi were present on apple at the time the various investigators made their observations, although to their knowledge they were dealing with only one.

SUMMARY

The assembled evidence indicates that the cultivated apple in eastern United States has been affected for some time by 3 distinct rusts, designated as "apple rust" (*Gymnosporangium juniperi-virginianae*), "hawthorn rust" (*G. globosum*), and "quince rust" (*G. clavipes*), of which the latter two, for the most part, were not recognized as distinct apple diseases by early workers, and that this condition probably accounts for much of the diversity of opinion as expressed in the literature relative to apple varietal susceptibility.

Comparative symptomatology of the 3 diseases and the morphological characters of their causative fungi are presented in tabular summaries as aids to diagnosis.

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THE RELATION OF AECIOSPORE GERMINABILITY AND DISSEMINATION TO TIME OF INFECTION AND CONTROL OF GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE ON RED CEDAR

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The studies reported herein were undertaken to clarify certain obscure phases in the life cycle of *Gymnosporangium juniperi-virginianae.*

AECIOSPORE GERMINATION

Workers with the rusts have long been familiar with the capricious germination of aeciospores.

While studying the effect of overwintering upon the germinability of aeciospores of *G. juniperi-virginianae* Schw. Miller[1] found that many remained viable and germinated well in March and April. This observation suggested the possibility of spring infection of red cedar. Using similar methods this study was repeated in 1933, 1934, 1935, and 1936. The results are presented graphically in figure 1. In general trend they agree with those previously reported but, as can be seen, there was considerable fluctuation in germination from month to month, especially in 1933, and there was some variability from year to year. The monthly fluctuations in 1933 may possibly be explained by the fact that the aeciospores tested for germinability in that year were secured by scraping them from a pustule with a scalpel. In subsequent years the aeciospores were dusted onto the slides by thumping the leaf with a finger, which probably released only apical, mature spores. No significant reason for the yearly variation in percentage of spore germination could be established from a study of the weather records for the years in question. There may be undetermined biological causes such as stage of maturity of the aeciospores and the amount of pigment in their walls at the time the leaves were taken from the trees. Cummins[2] suggested that the pigmentation of the walls of the aeciospores of certain *Gymnosporangium* spp. may be associated with an increased ability of the spores to remain viable during exposure to climatic extremes. Also, during certain times, especially warm periods, aeciospores have been found associated with other fungi, which may have influenced their germination.

AECIOSPORE DISSEMINATION

To obtain information on the time that the aeciospores are naturally blown to the red cedar, certain experiments were conducted. In June, 1934, noninfected potted seedlings covered with muslin tents were placed in the vicinity of apple trees bearing leaves and fruits heavily infected with *Gymnosporangium juniperi-virginianae.* These diseased leaves and fruits were allowed to fall and remain unmolested. The tents were removed from 3 trees during the entire month of July, then replaced and left on until the following June. This process was repeated with other trees, respectively, in August, September, October, November, December, January, February, March, April, and May, thus allowing 3 trees in each case to be exposed for a period of one month only, for each of the 10 months. In July, 1935, these 30 trees were labeled and put under similar conditions in the greenhouse and observed at regular intervals. In June, 1935, small galls were found on all of the trees that had been exposed in July, August, and September, 1934, and 2 galls were found on 1 tree exposed in October. No galls developed on any of the other trees. This experiment was repeated in 1935–1936, with similar results in that only trees exposed in July, August, September, and October became infected. Apparently, spore dissemination occurs in late summer and early fall.

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[1] Miller, Paul R. Pathogenicity of three red cedar rusts that occur on apple. Phytopathology 22: 723–740. 1932.

[2] Cummins, George Baker. Phylogenetic significance of the pores in urediospores. Mycologia 28: 103–132. 1936.

Cedar branches atomized with an aeciospore suspension in August were brought into the laboratory in February and washed with water. The washings were then lightly centrifuged, with the result that aeciospores were recovered and, when put under proper conditions, a few germinated. Apparently, some of the spores lie dormant on the red cedar leaf until growth actively begins in the cedar and the optimum temperature for germination is approached on the advent of spring.

TIME OF RED CEDAR INFECTION

In the fall and spring of 1929–1930, 150 inoculations were made on potted red-cedar seedlings placed in the greenhouse; 125 were inoculated October 1 and, the remaining 25, March 1. The method used was to atomize the seedlings with an aeciospore suspension. To maintain high humidity the inoculated trees were covered 3 days with bell jars. These jars were removed twice a day and the trees were sprayed with water. No infection was obtained with the fall trials. Three small galls formed on one tree inoculated March 1, but they did not develop to maturity because the leaves to which they were attached died. Some other leaves on the tree turned yellow and dropped off. The check trees, although treated the same way as the inoculated trees, did not show yellowing and dropping of scattered leaves; consequently, these symptoms may have been caused by rust infection. Trees used in this experiment were obtained from a nursery, and, although the majority of them had a bluish bloom, the particular one that bore the galls did not. It may be significant to note that in subsequent surveys natural infection on trees with bluish foliage has been looked for without success.

Fifty potted red cedars were inoculated with aeciospores from the same source in 1933 and 1934. Twenty-five were inoculated October 1 and, the remaining 25, March 1, with overwintered spores. Several galls were formed in early summer on one tree inoculated in the fall. Numerous galls were found on all of the trees inoculated in the spring. Symptoms appeared about

6 weeks earlier on the fall-inoculated than on the spring-inoculated trees. The galls on the former were much larger at maturity than were those on the latter. This relation between time of infection and size of resulting galls is being investigated further. Stained microtome sections of the galls showed that the galls developing on the fall-inoculated trees were attached to the stems, while those on the spring-inoculated trees were on the leaves.[3] Perhaps the explanation of this seasonal fluctuation in the loci of infection may be found in the fact that in the fall red cedar leaf activity is at a low ebb and possibly the stem tissue is then relatively more susceptible, whereas, in the spring, new succulent growth is present at the growing point of the leaf, which is at the base, where it forms the axil with the stem, in which presumably both spores and moisture accumulate.

A diagram of the life cycle of *Gymnosporangium juniperi-virginianae* as it now appears is given in plate III. The dates shown are, of course, generalizations—the exact times would depend upon the locality and season. As given, they apply fairly accurately to the commercial apple-growing regions in the Eastern States, except the most northern ones.

CONTROL

The experiments previously discussed indicate that, while aeciospores are blown to the cedar in late summer, they cause relatively few infections at that time, and that most of the infections occur after the spores have been overwintered, presumably in the axils of the leaves. Accordingly, quite contrary to previous assumptions, the disease is probably chiefly initiated in the spring, although some of the infection takes place in the fall. Naturally, the next step is to find whether a fungicide, applied during the dormant season, would prevent the overwintering spores from germinating and thus afford partial control of the disease. This the writer has had opportunity to do only in a very preliminary way. Six red cedar seedlings were inoculated July 5, 1936, by atomizing them with an aeciospore suspension. Trials made from a sample of these spores showed that about 1 per cent of them were capable of germination at that time. On January 10, 3 of these trees were sprayed with flotation sulphur, 5 pounds to 100 gallons of water. The remaining 3 trees were not sprayed. In June, 1937, these trees were observed for the presence of galls. The average number of galls on the nonsprayed trees was 17, and that on the sprayed trees was 2. Further investigation is needed before definite conclusions can be drawn as to the effectiveness of this control procedure, but, apparently, it should be an effective supplementary measure, applicable to ornamental species of *Juniperus* which, because of their special aesthetic value, it is desirable not to destroy.

Heald[4] conducted spray experiments on red cedar with 6–6–48 Bordeaux in Nebraska in the summer of 1907 at intervals between July 26

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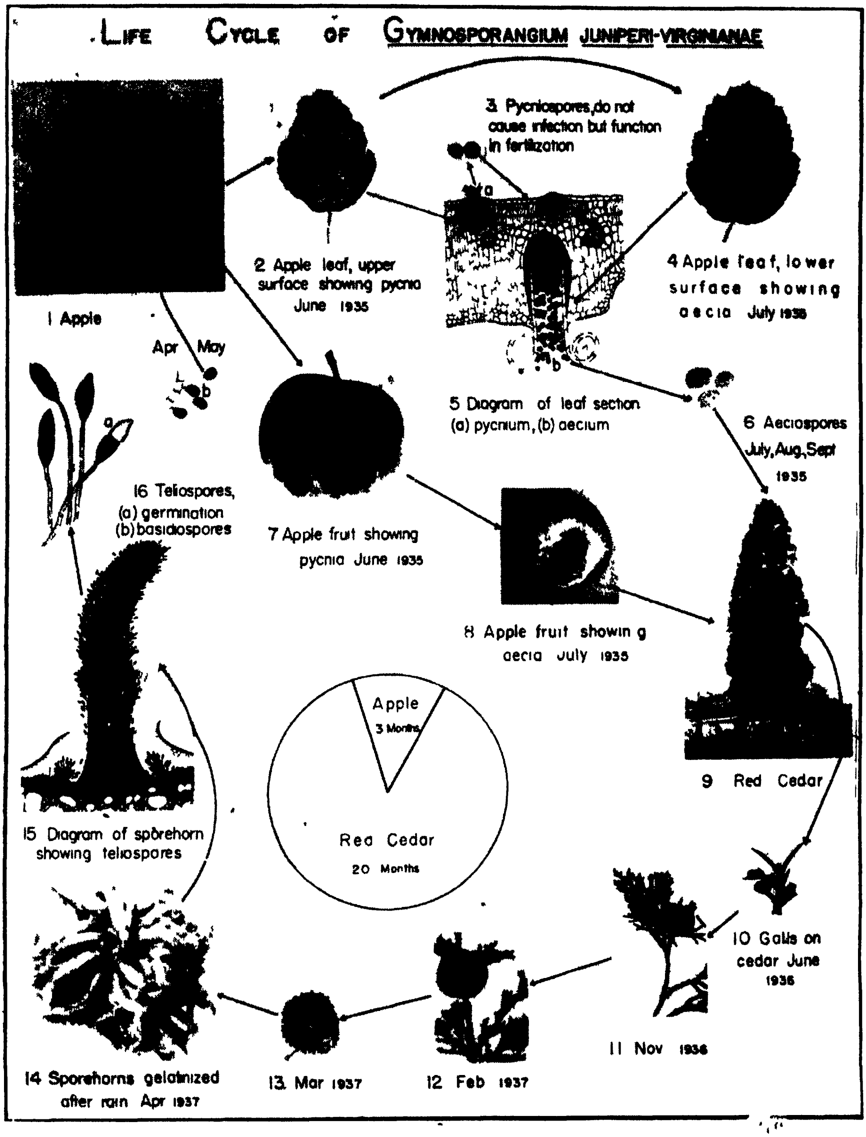
LIFE CYCLE OF GYMNOSPORANGIUM JUNIPERI-VIRGINIANAE
1 Apple
2 Apple leaf, upper surface showing pycnia June 1935
3 Pycniospores, do not cause infection but function in fertilization
a
4 Apple leaf, lower surface showing aecia July 1935
5 Diagram of leaf section (a) pycnium, (b) aecium
b
6 Aeciospores July, Aug, Sept 1935
7 Apple fruit showing pycnia June 1935
8 Apple fruit showing aecia July 1935
9 Red Cedar
10 Galls on cedar June 1936
11 Nov 1936
12 Feb 1937
13 Mar 1937
14 Sporehorns gelatinized after rain Apr 1937
15 Diagram of sporehorn showing teliospores
16 Teliospores, (a) germination (b) basidiospores
a
b
Apr
May
Apple 3 Months
Red Cedar 20 Months

PLATE III. Successive stages in the life cycle of *Gymnosporangium juniperi-virginianae* are as follows: Telial sori (sporehorns) are produced from galls on the cedar in the spring (14, 15, and 16). The teliospores embedded in the [illegible] germinate during spring rains (15 and 16) producing promycelia with secondary [illegible] (basidiospores) (16, b). The basidiospores are abjointed from their sterigmata and are blown away by the wind to the immature leaves or fruits of the apple where they germinate and produce infections resulting in pycnia on the fruit (7) and on the upper surface of the leaves (2), followed later by aecia on the lower surfaces of the leaves (4) and in very susceptible varieties on the fruits also (8). The aecia produce the aeciospores which are released and blown to the cedars chiefly during July, August, and September (6). Some of these germinate in the fall and the resultant mycelium remains dormant during the winter, while others remain in the axils of the twigs and do not germinate until early spring. The cedar host responds by the formation of characteristic galls (10, 11, 12, 13, and 14), thus completing the cycle in about 23 months during which the fungus is on the apple for only about 3 months. Numbers 5 and 15 are after Reed and Crabill, Virginia Agr. Exp. Stat. Bull. 9. 1915. Number 9 is a U. S. Forest Service photograph.

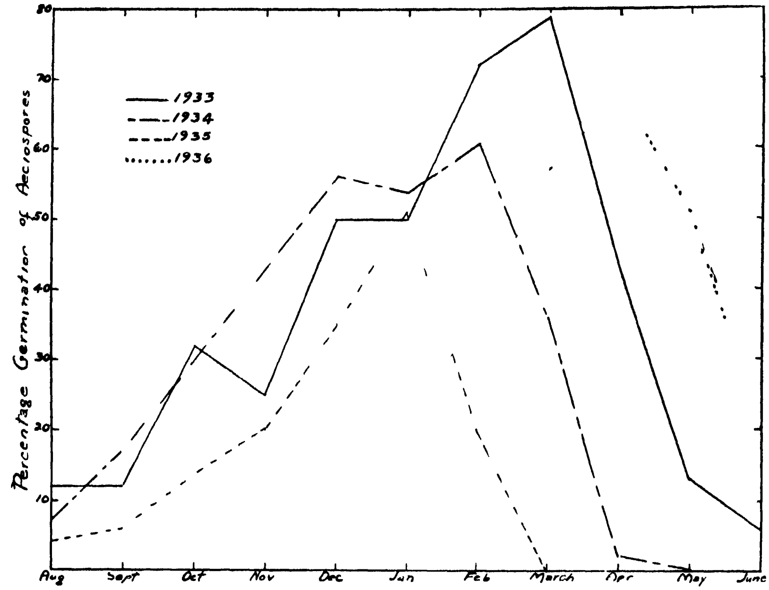

FIG. 1. Percentage germination of aeciospores of *Gymnosporangium juniperi-virginianae* at 24° C., as determined in tests made monthly from August to June, inclusive, for the years 1933, 1934, 1935, and 1936.

and October 9 and noted the effect upon the average number of galls developing in 1908. He reported that "Spraying at intervals from the period of maturity of the aecia to September 1 greatly reduced the number of 'cedar apples' " but in one block of trees upon which spraying was started September 2, practically no reduction in the number of galls resulted. MacLachlan and Crowell[5] obtained 90 per cent control through 4 applications of sulphur fungicides at intervals of 4 weeks, the first being made prior to the initial discharge of aeciospores. Although these investigators did not apply a dormant spray as such, in reality their applications may have acted as one. They say, "Examination of sprayed red cedar revealed that particles of the spray ingredient were still present in the axils formed by the leaves, six months after the last spray application." Their results indicate the possibility of control through one application of a dormant spray which would prevent the germination of overwintering aeciospores and thus inhibit spring infection of the red cedar, although their discussion does not show that they themselves had reached such a conclusion.

SUMMARY

Tests of aeciospore germination of *Gymnosporangium juniperi-virginianae* made monthly, from August to June, inclusive, over a period of 4 years, showed that the percentage germination in August was low and that maximum germination was reached at some time during late winter, varying from year to year. It was ascertained that dissemination of aeciospores occurs chiefly during July, August, and September.

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[5] MacLachlan, J. D., and Ivan H. Crowell. Control of the *Gymnosporangium* rusts by means of sulphur sprays. Journ. Arnold Arboretum 18: 149–163. 1937.

Experimental evidence showed the likelihood that there are 2 rather distinct periods when the red cedars become infected, first soon after the aeciospores are released when only a small percentage of them will germinate, and second, later in the season, possibly in early spring, when infection is caused by aeciospores that have overwintered on the *Juniperus* foliage prior to their germination.

Theoretically, an application of a fungicide to junipers during the dormant season offers protection against overwintering aeciospores and preliminary spraying experiments give promise of an effective control measure applicable to susceptible plantings of ornamental value.

DIVISION OF MYCOLOGY AND DISEASE SURVEY,
BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE,
WASHINGTON, D. C.

CRAZY TOP OF CORN

BENJAMIN KOEHLER

(Accepted for publication April 4, 1939)

A striking abnormality of corn, *Zea mays*, that apparently should be classified as a disease has been occasionally observed here and there at widely separated points in Illinois. The general appearance of the disorder and its occurrence at and above the ear shoots has suggested the name "crazy top." Marked losses from the disease have been observed in restricted areas.

Outside Illinois, crazy top has been reported to the writer by G. H. Dungan, University of Illinois, who observed it near Lizton, Indiana, in 1924; G. M. Smith, Purdue University, who observed it at several locations in Indiana more recently; and J. R. Holbert of Funk Bros. Seed Company, who observed it near Dana, Iowa, in 1938. A short report on the disease was made by Koehler and Holbert in 1930.[1] Since then it has been observed in a number of different hybrids as well as in open-pollinated varieties. Fully a dozen reports of its occurrence in Illinois were received by various staff members of the Agronomy Department, University of Illinois, in 1938. This was the largest number yet received in one year. Unfortunately, however, as heretofore, the disease was not noted until at or near harvest time. For that reason studies in the immature stages of plant development were impossible. Some new aspects of the disease were observed in fields studied in early October.

The principal characteristic of crazy top is the partial or complete absence of floral organs and their replacement by vegetative shoots (Fig. 1). Abnormalities of this type in corn have been reported following infections

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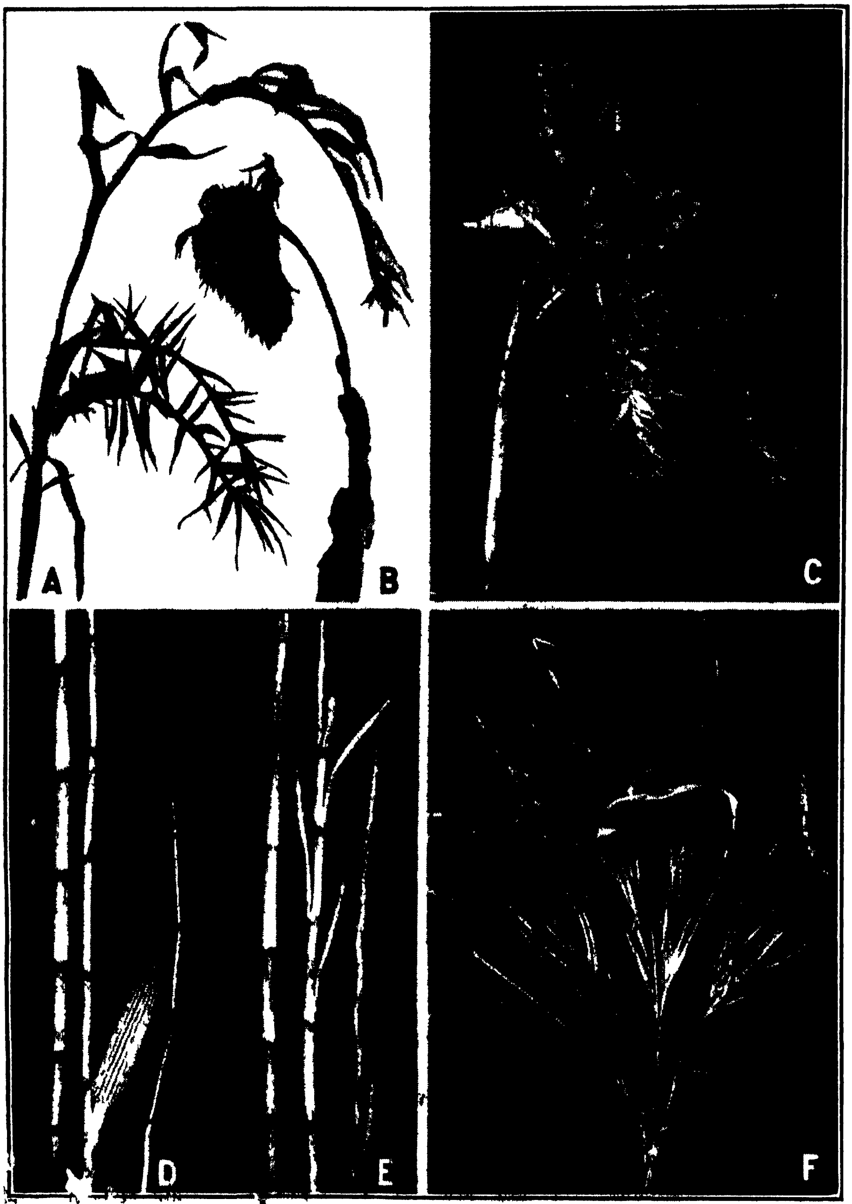
[1] Koehler, Benjamin, and J. R. Holbert. Corn diseases in Illinois, their extent, nature, and control. Ill. Agr. Exp. Stat. Bull. 35[illegible]. 1930.



Fi[illegible]. Cra[illegible] op plant with many jointed branches in place of ear shoots, and excessively joint[illegible] the main stalk. B. Crazy-top plant with tassel converted into a large [illegible] Thi[illegible] the same general disorder as in A, but the causal agent pr[illegible] n the development of the plant. C. Close-up of a veget[illegible] A com[illegible]te normal plant of Illinois Hybrid 960, except that all leaves w[illegible] Th[illegible]e hybrid affected with crazy top of the character seen in A, showing total [illegible] odes and some variations from A in the number and length of side branches [illegible] foxtail showing excessive branching and shortened internodes, found in the [illegible]ere crazy top of corn occurred abundantly.

with *Sorosporium* and *Sclerospora,* but the disease here reported is doubtless of a different nature. In cases of partial replacement by vegetative shoots, one may find the tassel deranged but the ear shoot bearing grain, and in such cases one may find among the leafy proliferations of the tassel a few branches bearing apparently normal male florets. The large bunchy vegetative growth replacing the tassel (Fig. 1, B, C) is one of the most pronounced characteristics of the disease. In one hybrid field, however, nearly half of the abnormal plants did not have this bunch at the top but, instead, the upper half of the stalk was excessively jointed (Fig. 1, A, E). Some examples of the same symptoms also were found in a field grown from open-pollinated seed.

No apparent difference between normal and crazy-top plants was found with respect to the number of nodes between the ground level and the first ear shoot. Furthermore, in plants bearing a bunched top, regardless of whether they bore normal ears or abnormal leafy branches, the total number of nodes from soil level to tassel joint was about normal. In the other type of expression of crazy top (Fig. 1, A, E) the number of nodes above the top ear branch averaged 23.6 in a certain field studied, whereas, in normal plants, the number of nodes from the top ear to tassel was 6.2. Such plants never bore ears but always produced leafy branches in their place. These branches ranged from one to many per plant and varied greatly in length; some were even longer than those shown in figure 1, A. The number of nodes per leafy branch averaged about 23.

The occurrence of crazy top in a field was always spotted, the spots coinciding consistently with depressions in the field. In some cases the depressions were only very slight. These areas of low elevation usually were provided with good facilities for drainage, and, after heavy rains, water would be expected to stand there for only a short time. The observed extent of damage varied from an approximate 60 per cent loss in a 4-acre field planted to open-pollinated corn to only a few specimens in some other cases. About 5 acres were involved in a large field growing hybrid corn. In the central part of this area 71 per cent of the plants were abnormal and for the most part produced no grain. The total loss to the corn crop of Illinois, however, was negligible. Crazy top was found on moderately, as well as on highly, fertile brown and black soil in the northern half of Illinois, and also on less fertile, gray soil in the southern part of the State.

These malformations have been considered attributable to a disease rather than a heritable morphological aberration, because of its spotted occurren in various different open-pollinated varieties and hybrids or seasonal variations in prevalence. The cause of
It obviously operates before the differentiati
In case of tassel malformations this would be
Illinois, when the uppermost grov
than a few inches above the soil leve

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[2] Bonnett, O. T. Development of the stam
corn, *Zea mays saccharata.* Manuscript in preparation

at the most, extend to 20 or 30 inches above the ground. In cases where no bunched top was formed, but only an excessively jointed stem, the disease was active still earlier, before tassel initials formed, which is before the growing point extends above the soil surface.

An interesting side light is that in one of the severely diseased corn areas, barnyard grass, *Echinochloa crusgalli,* and green foxtail, *Setaria viridis,* were excessively branched and presented a very abnormal appearance (Fig. 1, F). The particular areas where the malformations in the 3 kinds of plants occurred coincided markedly.

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PHYTOPATHOLOGICAL NOTES

Physiologic Strains of Bean Rust.—In the course of a project for the development of snap-bean varieties resistant to bean rust, collections of rust material were obtained from commercial fields in Florida and Washington. The 2 physiologic races of bean rust described by Harter[1] [2] were also obtained from him. The testing for rust was done on plants in the field, on plants in the greenhouse, and in Petri dishes. In the dish method abscised leaflets, floated bottom-side-up on a 5 per cent sucrose solution, were inoculated by brushing with urediospores. The dishes were kept in the light at room temperature. The reading was made 12 to 14 days after inoculation. The 3 methods of testing gave almost identical results for all varieties tested, indicating that the dish test is reliable.

The rust material from the Florida and Washington collections was propagated repeatedly on a single-pustule basis, in dishes, to insure that only pure strains were being used. There was some evidence of more than one strain present in the original Florida collection, but only one strain was propagated. The Washington collection gave no evidence of containing

TABLE 1.—*Reactions of 4 strains of bean rust on 3 differential hosts*

Bean varieties	Strains of rust			
	1[a]	2[a]	W[b]	F[c]
Brown Kentucky Wonder No. 928	0	0	7	0
Tennessee Green Pod	10	10	10	2
Golden Gate Wax Pole	0	8	0	0

[a] Obtained from Dr. L. L. Harter, U. S. Horticultural Station, Beltsville, Maryland.

[b] Obtained from Kale Canning Co., Everson, Washington.

[c] Obtained from Dr. G. R. Townsend, Everglades Experiment Station, Belle Glade, Florida.

to the Washington strain (W), while Golden Gate Wax is resistant to strains 1, W and F, but susceptible to form 2, and Tennessee Green Pod is resistant to strain F, but highly susceptible to 1, 2 and W. It is, therefore, evident that the rust strains obtained in Florida and Washington collections differ from each other and from the two previously described strains 1 and 2.—B. DUNDAS and G. W. SCOTT, Pacific Coast Breeding Station, Milpitas, Calif., and Associated Seed Growers, Inc., New Haven, Conn.

The Use of Special Media for Sporulation of Fungi.—Leaf juice expressed from *Platanus racemosa* leaves and sterilized by filtration through Chamberlain filters has been used successfully in producing sporulation of leaf-spot fungi (*Stigmella platani-racemosae* Dearn. and Barth., *Stigmina platani* (Fckl.) Sacc., *Mycosphaerella platanifolia* (Cooke), and *M. stigmina-platani* Wolf). The natural juice thus obtained was placed as drops in van Tieghem cells and as drops on slides supported in Petri dishes by means of U-shape glass rods. The medium, little of which is needed, lasts indefinitely if contaminants be excluded and the material be kept cold in tight-stoppered, sterile bottles. Spores grown on this medium and those produced on the living host are identical.

Abundant spermatia have been formed by the above-named fungi on sterile filter paper in tubes to which the medium was added aseptically. The use of this host juice offers a means of suggesting whether a perfect stage may be expected in these fungi and indicates that their perfect stages can be formed by the latter method if other conditions are favorable.

Natural media can be obtained from the host or host part desired and may prove successful for sporulation of certain other fungi that do not sporulate well on ordinary media.—DONALD J. SMITH and C. O. SMITH, University of California Citrus Experiment Station, Riverside, California.

A Destructive Bud-transmissible Disease of Sour Cherry in Wisconsin.—An unfruitful condition of sour cherry (*Prunus cerasus* L.) that has been called "boarder tree," "yellow leaf," or "physiological yellow leaf" is widespread and increasing in Wisconsin. Affected trees tend to have relatively large leaves, some of which develop conspicuous chlorotic areas. The chlorotic leaves and some that are still green are abscised, a major wave of defoliation usually occurring in late June or early July. The spur system becomes greatly reduced and the crop very sparse.

Reciprocal budding experiments between diseased and healthy Montmorency trees resulted in transmission of the disease in cases where a union of diseased and healthy tissues occurred. Characteristic leaf symptoms of the disease developed in 1939 on shoots from healthy buds that were placed on diseased trees in 1938. Similar symptoms appeared on shoots from diseased buds on healthy trees. Leaves on many shoots of previously healthy trees on which buds from diseased trees had been propagated showed the characteristic symptoms. Controls showed no symptoms of the disease.

Microscopic examinations and platings from diseased organs have given no evidence of a causal fungus or bacterium.

The evidence herein reported indicates that the disease is caused by a virus. Further studies of the disease and of its possible relations to previously described virus diseases are in progress.—G. W. KEITT and C. N. CLAYTON, University of Wisconsin, Madison, Wisconsin.

REPORT OF THE 1939 ANNUAL MEETING OF THE PACIFIC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The twenty-third annual meeting of the Pacific Division of The American Phytopathological Society was held at Stanford University at Palo Alto, California, from June 27 to 30, 1939, in conjunction with the meetings of the Pacific Division of the American Association for the Advancement of Science. Thirty-two papers, reporting research projects, were presented during 4 half-day sessions. A total of 70 pathologists and others interested in the subjects attended the sessions.

Thursday morning was devoted to a symposium on teaching of Plant Pathology. Specific phases of the problem were discussed by W. W. Robbins, J. T. Barrett, W. B. Hewitt, H. R. Stanford, and T. E. Rawlins.

A field trip of unusual interest, consisting of visits to several greenhouses, commercial plantings of ornamentals between Palo Alto and San Francisco, and a tour through one of the large estates occupied all of Friday. An opportunity was furnished to observe the culture and diseases of asters, chrysanthemums, gardenias, roses, and a number of other ornamental plants.

Officers of the society for the ensuing year are as follows: President, B. F. Dana, U. S. Department of Agriculture, Corvallis, Oregon; Vice-president, T. E. Rawlins, University of California; Secretary-Treasurer, L. D. Leach, University of California, Davis; Councilor, E. Carsner, U. S. Department of Agriculture, Riverside.

The next annual meeting will be held at the University of Washington, Seattle, Washington, in June, 1940.

Titles and abstracts of papers presented at the meeting follow.

L. D. Leach,
Secretary-Treasurer.

Overwintering mycelium of Plasmopara viticola (B & C) Berl. & DeT. in the California Wild Grape, Vitis californica Benth. [illegible] T. BARRETT. In 1904, Istvanffi reported finding mycelium in the [illegible] grapes [illegible] left on the vines during the winter, and in inner bud scales in December. The la[illegible] [illegible] an observation of Cuboni. These and other students of downy mildew have suggested [illegible] a method of overwintering on cultivated grapes, but no recorded proof has been found. In California it has been established that mycelium, resting over winter in the cortical [illegible] and buds of the canes, is a regular method of downy mildew perpetuation in the wild grape, *Vitis californica* Benth. Shoots that develop from infected buds are conspicuously more vigorous than those from noninfected ones. Many measurements made throughout the growing season show the diseased shoots 2½ times longer than healthy ones and twice the diameter at the base. Mycelium is easily demonstrated microscopically in the parenchyma of all organs, even to the very tip of growing points. Diseased shoots are conspicuous by their paler leaves and stems. No sporangiophores have been observed in nature on parts other than leaves where they appear at first only along the veins on the lower surface. Rarely, except for leaves, is there any killing of infected parts. Leaf spots produced by secondary infections are scattered at random and soon become yellow and finally brown. This type of infection is greatly dependent on the weather. Infection of the cultivated grape, *Vitis vinifera* L., even in close proximity to severely infected wild grapes, has not been observed, a fact that may be largely dependent on unfavorable climatic conditions.

Pathogenicity and Pathological Histology of Phymatotrichum omnivorum in a Woody Perennial, the Pecan. LLOYD A. BRINKERHOFF. Roots of pecan trees showing earliest symptoms of infection were found extensively injured. The so-called resistance of pecan to root rot is not attributable to its ability to resist invasion. Infected trees may survive as long as 2 years by means of shallow roots that have escaped infection. The ability to produce abundant adventitious roots following treatment is a major factor in the recovery of infected trees. Infection was secured readily on roots of all sizes above ⅛ in. diameter. In August, lesions appeared in 9 days from inoculation, and the average rate of spread along roots during July and August was from .6 to .8 in. per day. Primary spread of the fungus is by strands on the surface of the root. Initial penetration was found to be most rapid through lenticels, but occurs also through the point of emergence of lateral roots and through normal breaks in the periderm. After initial penetration, lateral spread is rapid through tissues just under the periderm, and through the cambial region. Radial penetration is largely through the pitted phloem and xylem ray cells. Starch disappears rapidly from invaded cells. Granular deposits composed of suberin or suberin-like substances were abundant in the zone of infection.

Spraying by Airplane for Disease Control in Peach Orchards. P. D. CALDIS. Applications of spray materials for the control of peach diseases by airplane have been made on a considerable scale since 1936. A suspension of a copper fungicide, with or without lead arsenate, in summer, or winter spray oil, is atomized at the rate of 10 gal. per acre, covering 50 acres per hour. One package Bordo, Coposil, Cuprocide, and Basicop, have been successfully used. The deposits adhere and weather better than do water sprays. The control obtained is similar to applications by power ground machines, although the cost is slightly higher at present. Under wet soil conditions in the spring, when ground spraying is impossible, timely applications can be made without packing the soil and tramping down the cover crop.

Morphological and Anatomical Features of Phyllody in Varieties of Tomato and Bean. B. F. DANA. Phyllody was observed in the Pacific Northwest on varieties of common, Lima, and soy beans, tomato, squash, and carrot, many of which also showed curly-top injury. Phyllody resulted in change of floral parts to leafy structures and extension of the axis between those whorls of the flower where vascular traces for the different whorls are known to be separate. In common bean secondary or accessory phylloid flowers, inflorescences with phylloid flowers, and shoots were formed. In common bean, transformation of the carpels ranged from an expanded sack-like structure through a marginal-veined leaf-form with marginal leaflets representing ovules to a normal-appearing leaf. Phyllody in tomato was accompanied by proliferation in the region of the internal phloem similar to that described for the big-bud disease of tomato. In pedicels of phylloid flowers of common bean and soy bean pith tissues were filled with starch, a feature described as characteristic of big bud and woodiness on tomato and bindweed. Many plants bearing phylloid flowers also exhibited symptoms of curly top. Further study will be necessary to verify the presence of the big-bud virus and to determine the influence of the curly-top virus in the production of phyllody in these hosts.

The Effects of Sea Spray Deposits on Spore Germination and Mycelial Growth of the Cypress Canker Fungus. A. W. DIMOCK. Experiments to determine whether or not [illegible] spray deposits on the bark and foliage of *Cupressus macrocarpa* in the native groves along the immediate coast might provide protection against the cypress canker fungus, *Coryneum cardinale*, yielded the following information. Salt deposits of [illegible] as 335 mg. of chloride per 100 sq. cm. were present on old [illegible] cypress trees on the immediate ocean front but deposits were only 1/6 to [illegible] as heavy on young, infection-susceptible bark of the same trees and only 1/11 to 1/1[illegible] as heavy on foliage. Growth of mycelium of *C. cardinale* occurred in salt solutions [illegible] 35 per cent of saturation (12–14 per cent sodium chloride). Spores of the fungus remained viable after suspension for as long as 5 days in saturated solutions of sodium chloride. Spore germination, averaging 72–84 per cent in check tests in distilled water, was reduced to 0–4 per cent by films from sea water on glass slides and significantly by sodium chloride films. On cypress bark and foliage, however, natural and artificial deposits of sea spray and of sodium chloride solution either caused no reduction in germination or the reduction was so variable that a protective effect could scarcely be counted on.

Host Range and Strains of the Powdery Mildew (Erysiphe polygoni) of Bean and Cowpea. B. DUNDAS. Isolations of powdery mildew (*Erysiphe polygoni*) from *Phaseolus* and *Vigna* species have been compared as to their pathogenicity on about 250 varieties of *Phaseolus, Vigna* and related genera by inoculating living detached leaflets supported on a 10 per cent sucrose solution in Petri dishes. In maintaining the isolations, the leaflets were kept 4 days in the dishes before inoculation to insure against volunteer mildew contamination. Mildew on beans and cowpeas was collected from various parts of California, from Florida and Michigan. Fourteen different strains have been isolated. These are readily separated into 4 classes on the basis of their reaction on beans and cowpeas; 3 of the classes contain only 1 strain each, the fourth embracing the other 10. These 10 are differentiated mainly on species of wild beans, *Dolichos,* and *Cyamopsis.* Inoculations of mildew from beans and cowpeas on various species of *Lathyrus, Trifolium, Medicago, Lotus, Melilotus, Trigonella, Brassica,* and several other genera produced no infection. Likewise, mildew from these species did not infect beans and cowpeas. The forms of *Erysiphe polygoni* occurring on *Phaseolus* and *Vigna* are limited mainly to these genera.

Inheritance of Resistance to Powdery Mildew in Runner Beans (Phaseolus coccineus), Tepary Beans (P. acutifolius), Yard Long Beans (Vigna sesquipedalis) and Cowpeas (Vigna sinensis). B. DUNDAS. Within the runner beans, tepary beans, cowpeas and yard long beans were found varieties both resistant and susceptible to various strains of powdery mildew (*Erysiphe polygoni*) of beans and cowpeas. Crosses were made between resistant and susceptible varieties and have been carried through the F_2 generation. The dish method of testing was used. All F_1 plants from these crosses between resistant and susceptible plants were resistant, while the F_2 populations segregated in a ratio of 3 resistant to 1 susceptible. These results indicate that the resistance to strain 1 in runner beans, to strain 2 and 3 in the tepary variety "Gray Mottled", in the cowpea variety Blackeye and in yard long bean is due to a single dominant Mendelian factor. Furthermore, the individual F_2 plants of tepary and *Vigna* reacted the same to both strain 2 and 3, which shows that the reaction to both strains is controlled by the same factor. The F_2 from a cross between a resistant Blackeye and a resistant Yard Long gave only resistant progeny, showing that both contain the same factor for resistance.

Hydroxyl ion Concentration of the Saliva of Partially Desiccated Beet Leaf Hoppers. J. M. FIFE.

Additional Celery Viroses. JULIUS H. FREITAG and HENRY H. P. SEVERIN. The name "pseudo-calico" is proposed for an additional virosis of celery. The symptoms of 6 celery viroses occurring naturally in California and 2 viruses that have been experimentally transmitted to celery have been previously described. The celery pseudo-calico virus has not been transmitted by any of the 9 species of aphids that breed on celery. Celery crinkle-leaf, poison-hemlock ringspot and celery yellow spot are transmitted by aphids. Crinkle-leaf and the ringspot viruses were retained by the aphids for periods of less than 24 hours, while yellow-spot virus was retained by the honeysuckle aphid (*Rhopalosiphum melliferum*) for 12 days. Pseudo-calico and crinkle-leaf viruses are transmitted by sap inoculation, but yellow-spot has not. Ringspot virus has been transmitted by mechanical inoculation with difficulty from plain parsley to plain parsley, but it has not been transmitted by this method to celery. The physical properties of some of these viruses were as follows: pseudo-calico, thermal inactivation 70° C., dilution tolerance 1–1000, and longevity *in vitro* 5 days; crinkle-leaf, thermal inactivation 60° C., dilution tolerance 1–100, and longevity *in vitro* 3 days. Pseudo-calico virus, up to the present time, has been transmitted to 12 species of plants in 5 families. The host ranges of crinkle-leaf and ringspot viruses are limited to plants belonging to the family Umbelliferae.

Camellia Blossom Blight. H. N. HANSEN and H. EARL THOMAS. A destructive blight of the flowers of Camellia (*Camellia japonica* L.) was first observed in 1938 in one nursery in the San Francisco Bay region. Serious outbreaks usually follow each of the rather frequent rains from January to late April. All varieties of *Camellia* seem to be equally susceptible to this disease. The first symptom of the disease is the appearance on the petals of small brownish specks that enlarge rapidly and unite into large patches eventually involving the entire flower, which soon drops and becomes dark brown. The causal organism is an undescribed species of *Sclerotinia,* apparently specific to the floral parts of *Camellia.* A distinguishing character of this fungus is its black, laminated sclerotia, which, in shape, typically conform to the petal arrangement of the flower. The sclerotia apparently are formed in the flowers only, and after one or more years of rest they give rise to apothecia. In mass the non-viable microspores are jet-black. There are no secondary spore forms. Inoculation produced typical symptoms in less than 36 hours. The nature and behavior of the pathogen suggest that destruction of fallen flowers would effectively control the disease.

Registration of Citrus Trees Inspected for Psorosis. J. LEE HEWITT. There is a registry of citrus trees selected by California nurserymen to serve as sources of cion material and examined by the State Department to determine their probably entire freedom from infection with psorosis. To date, there are registered 381 Valencia orange trees, 38 Washington Navel orange trees and 1 tree called Seedless Valencia. There are 63 nurserymen whose applications for registration include varieties of orange, lemon, tangerine, grapefruit and seedling orange trees. This registry is based upon 5 assumptions: 1. Psorosis is largely bud transmitted; 2. It is visibly detectable; 3. Inspection being upon voluntary request, there is no compulsion upon the department immediately to declare as to eligibility; 4. The public will demand trees known to be from registered bud sources; 5. The nurserymen will devise means to meet the public demand. The biological conditions for registration specified by Howard S. Fawcett includes two essentials: trees shall be older than 15 years or probably derived from older trees that are available for inspection; examination in good season of the bark and of the leaves at 15 stations on each tree shall reveal no suspicion of psorosis. The State makes no guarantee and writes no certificate, except a letter to the applicant transmitting a copy of the registry entry, which includes a detailed description of the location of each tree.

Transmission of Black-raspberry Mosaic by the Cane-feeding Aphid, Amphorophora rubicumberlandi. GLENN A. HUBER. A cane-feeding aphid, *Amphorophora rubicumberlandi,* has recently been found on both wild (*Rubus leucodermis*) and cultivated (Cumberland var.) black-raspberry plants in the Puget Sound area in western Washington. In greenhouse studies, *A. rubicumberlandi* transmitted black-raspberry mosaic from the wild black raspberry to 30 per cent of the plants of cultivated black raspberry (Cumberland var.) and from mosaic Cumberland to 50 per cent of the plants of the same variety. Active colonies are difficult to maintain on mosaic-infected plants. In the field, they feed only on large, rapidly growing canes. They have not been observed feeding on leaves, and seldom within 10 to 12 inches of the growing tips. *A. rubicumberlandi* has been observed feeding and reproducing on the following varieties: Black Pearl, Bristol, Conn. 96, Conn. 129, Cumberland, Dundee, Evans, Mitch seedling, Munger, Naples, New Logan, Ohio, O.S.C. 106, Plum Farmer, Quillen, and Shuttleworth. It has not been found on Black Beauty, Harbert, 2 Kansas seedlings, 2 O.S.C. seedlings, or purple cane varieties. All attempts to make it feed on varieties of red raspberry have failed.

The Occurrence of Sclerotinia fructicola and S. laxa on Stone Fruits in Western Washington GLENN A. HUBER and KARL E. BAUR. *Sclerotinia fructicola* and *S. laxa* were found responsible for brown rot of stone fruits in western Washington. Results of isolations follow: APRICOT. *S. laxa* from blighted twigs and cankers, infected blossoms and decaying fruit. CHERRY (sweet and sour varieties). Both species from infected blossoms and decaying fruit. *S. laxa* from twig cankers and conidia on mummies overwintering on the trees (8 to 46 per cent showed sporodochial production from December through April). PEACH. Both species from decaying fruit and mummies overwintering on the trees. *S. laxa* from infected blossoms and twig cankers. PRUNE. *S. fructicola* and *S. laxa* in the proportion of 20 to 1 from mature fruit. *S. fructicola* from apothecia. *S. laxa* and *S. fructicola* from mummies overwintering on trees. (Zero to 11 per cent produced viable brown rot conidia of which less than 2 per cent produced *S. fructicola.*) *S. fructicola* from 93 per cent of blossoms blighted from other causes; 50 to 100 per cent from surface of normal blossoms. *S. fructicola* from 0 to 92 per cent of the thrips collected from normal blossoms in various orchards and placed on culture media. One orchard showed 92 per cent of thrips carrying spores of *S. fructicola.*

The Effect of Calcium Cyanamid on Development of Apothecia of Sclerotinia fructicola and on Population of Taeniothrips inconsequens in Prune Orchards. GLENN A. HUBER, KARL E. BAUR and EDWARD P. BREAKEY. Further studies conducted in prune orchards of Clark County, Wash., during the spring of 1939 showed that commercial pulverized and oiled calcium cyanamid applied to the surface of the soil and vegetative cover at the rate of 300 pounds per acre prevented development of apothecia of *Sclerotinia fructicola* and reduced the number of thrips, *Taeniothrips inconsequens,* that emerged from the soil. Duplicate areas were treated in orchards in 3 localities within the period March 3 to 27. Replicate apothecial counts were made on the treated and nontreated plots at intervals during the period of apothecial development, April 1 to 20. Apothecia developed on the nontreated areas (7–40 per measured area of 36 sq. ft.), while none developed on the treated plots. The average total number of thrips that emerged, as determined by trap (each covering 9 sq. ft.) counts, on nontreated areas in the 3 orchards was 708.5, 133.5, and 48.5, respectively, and on the treated plots was 79, 13.5, and 5.5, respectively.

Virus Concentration in Root Tips of Resistant and Susceptible Sugar Beets. C. F. LACKEY. In previous experiments root tips were obtained from sugar beets infected with field virus. This report gives data from experiments with the resistant variety 68 and a susceptible commercial variety. The virus used was curly-top strain number 1. Twenty tips each 5 mm. long were cut from each beet variety, mascerated, and fed to nonviruliferous leaf hoppers in each experiment. Test beets infected by these vectors indicate a higher concentration of virus in the resistant beet root tips than in those of the susceptible variety.

Spread of Armillaria in a Peach Orchard Over a Period of Ten Years. L. O. LAWYER.

Soil Fumigants for the Commercial Control of Armillaria. L. O. LAWYER, HAROLD E. THOMAS and P. D. CALDIS.

Breeding for Resistance in Blackeye Cowpeas to Cowpea Wilt, Charcoal Rot, and Root-Knot Nematode. W. W. MACKIE. Blackeye beans, or cowpeas (*Vigna sinensis*), in California have deteriorated in yield because of increasing ravages of cowpea wilt (*Fusarium vasinfectum tracheiphilum*), charcoal rot (*Rhizoctonia bataticola*), and root-knot nematode (*Heterodera marioni*). Escape lies in the breeding of resistant varieties. The Iron cowpea has proved resistant to cowpea wilt and root-knot nematode since the classical work of Orton and Webber, and is superior stock for breeding under intensive tests. By crossing the California Blackeye with Iron as the male parent, followed in the F_1 and subsequent generations by back-crossing to the Blackeye and concluded by plant selection, a number of varieties superior in yield, market quality, and disease resistance have been created. To achieve these results the genetical behavior of the factors involved were studied. In the F_1, resistance to the three parasites was found to be dominant. Late maturity, vigor and spread of vine, high yield, mottling, and self-color in seed are also dominant. The eyed factor is recessive, due to a single factor permitting the complete elimination of all except eyed forms.

The White Streak or White Stripe Disease of Narcissus. FRANK P. MCWHORTER. White streaking is a chief symptom of a transmissible narcissus disease, which presumably is a virosis. Mid-season symptoms usually consist of prominent white and purple longitudinal streaks on the leaves, but the symptomatology is extremely variable. In general, higher temperatures mask purpling and increase white streaking. The histological anatomy of the disease shows it is very different from yellow streak or mosaic. The white streaks are dead areas of sunken epidermal or chlorenchyma cells. The yellow or mosaic streaks are raised overgrowths of living cells. The two diseases are, therefore, easily distinguished when typically developed.

The Efficacy of Some Insoluble Copper Sprays for the Control of Walnut Bacteriosis in Oregon. P. W. MILLER. In studies carried on in Oregon from 1932 to 1938, inclusive, the following insoluble copper compounds have been tested in the field and their phytocidal effect and relative efficacy for the control of walnut bacteriosis determined: Cupra-ammonium carbonate, copper resinate, copper carbonate, basic copper sulphate, copper phosphate, copper oxalate, copper hydroxide, copper oxychloride, cupra-ammonium silicate, and copper zeolite. Only one of these, namely, copper oxalate, has given consistently good control of the disease. In 3 of the past 4 years this copper compound, at the rate of 3 to 4 lb. to 100 gal., has given better control of walnut bacteriosis than Bordeaux mixture 2-2-50. Unlike Bordeaux mixture, copper oxalate, at the above rate, has caused no detectable foliage injury under Oregon conditions. While the results of studies carried on to date are very promising, further testing under a wider range of local and seasonal conditions is necessary, however, before it can be concluded that copper oxalate is more efficacious for the control of walnut bacteriosis than Bordeaux mixture.

Delphinium Aster Yellows. HENRY H. P. SEVERIN and SIDNEY J. OLIVER. The mountain leaf hopper, *Thamnotettix montanus*, and the geminate leaf hopper, *T. geminatus*, are the most important vectors of the California aster-yellows virus to garden varieties of perennial delphiniums, and both species breed on this host plant under natural conditions. Adults of both species of leaf hopper were collected on naturally infected delphiniums and transferred to healthy delphinium seedlings. *T. montanus* transmitted the virus to 84.6 per cent and *T. geminatus* to 92.3 per cent of the plants. The recovery and transfer of the virus to healthy celery from naturally infected delphiniums by previously noninfective *T. montanus* was 20.8 per cent and *T. geminatus* 4.2 per cent. Experimental infection of healthy delphiniums with the virus by the two species of leaf hopper was as follows: seedlings and second-year delphiniums before the spikes developed, 100 per cent; after the spikes developed, 90 per cent. The incubation period of the disease in 4 varieties or hybrid delphinium seedlings infected during June varied from 15.4 to 28.0 days, average 19.5 days; in delphiniums infected during the second year before spikes developed, varied from 29.2 to 64.0 days, or an average of 43.5 days; and after spikes developed from 28.3 to 72.0 days, average 45 days. In the 3 experiments on the incubation period of the disease, the virus was recovered and transferred to healthy aster or celery plants from 10 of 92 experimentally infected delphiniums, or 10.9 per cent.

Culture Methods in Relation to Fusarium Identification. W. C. SNYDER and H. N. HANSEN. With the conviction that any taxonomic system must be based upon standardized practices in order to fulfil its purpose in serving all workers, a study has been made to determine the influence of various conditions of growth upon fungus characters used in the identification of *Fusaria.* Conditions of culture, which are reflected in important degrees in characters of growth obtained, include the purity of culture, kind of medium, method of transfer, frequency of transfer, past cultural history of the fungus, age of culture, amount of light in the environment, temperature, and variability of the fungus. It is suggested that failure to make effective use of the existing taxonomic scheme for the genus *Fusarium* is due in large part to a general lack of understanding of the cultural practices and technique upon which the system is based.

The Effect of Intercrops and Forage Crops on the Incidence and Severity of Phymatotrichum Root Rot on Pecan. R. B. STREETS. Intercropping or the pasturing of sheep or cattle on forage crops grown in Arizona pecan groves has proved a satisfactory source of revenue, but nearly all serious outbreaks of root rot have occurred with an intercrop of alfalfa. Alfalfa roots furnish a medium on which the root-rot fungus spreads very rapidly and infects the pecan roots. A 40-acre pecan orchard on infested soil and interplanted with alfalfa showed after 2 years 195 infected trees in comparison with 58 infected trees in an adjacent orchard on even more severely infested soil, or 3.4 times as many infected trees in the alfalfa. Removal of the susceptible intercrop does not result in an immediate drop in the number of newly infected trees. A 50-acre orchard from which the alfalfa was removed in August, 1937, showed 114 new cases of root rot in the pecan trees in June, 1938, but only 5 new cases or 4.4 per cent as many in June, 1939. The substitution of root-rot immune forage crops, such as barley, in winter, and Sudan grass, in summer, has already shown great promise by markedly reducing the incidence and severity of root rot. Other possible rotations are winter vegetables or clean fallow and grain sorghums in summer.

Further Studies on the Control of Phymatotrichum Root Rot in the Pecan by Soil Treatments. R. B. STREETS and LLOYD A. BRINKERHOFF. Maps of 590 acres of pecans in 5 groves have been made each June and November to record progress of root rot and effectiveness of control measures. In 1937, 301 infected trees received soil treatments, and 610 trees were treated in 1938. Ammonium sulphate or ammonium phosphate 16–20 applied in basins and leached into the soil by irrigation is usually sufficient to check the disease, but injection of solutions of either salt in combination with dilute sulphuric acid (1%) into the soil around the trunk has given the most rapid and certain response. Solutions and sulphur suspensions were injected to a depth of 4 feet by an injecting rod and power sprayer operating at 300 pounds' pressure. Three-year-old trees (4-inch trunk diameter) were injected at the rate of 50 per day by two men, demonstrating that the method is rapid and practical. Data taken in June, 1939, showed that two orchards, totaling 130 acres, in which all infected pecan trees were treated by various methods in 1937 and 1938 gave the following response: excellent condition, 150 trees; good, 114; fair, 25; poor, 22; and dead, 35. At least 25 trees died unnecessarily following failure to cut back the tops and water them sufficiently following treatment.

The Stem Nematode Disease of Oats and Peas. HAROLD E. THOMAS. For several years a disease caused by the stem nematode, *Tylenchus dipsaci,* has been responsible for the partial failure of oats in a very small area near Watsonville, California. Recently, canning peas of the Perfection variety have been severely affected in this area by the same nematode, when they follow oats. Injury to peas is noticeable soon after the plants come up. The terminal bud fails to extend and one or more side shoots may form successively. Very little swelling of pea stems has been observed. Three consecutive years of cropping to peas failed to reduce the injury in a succeeding oat crop. Affected oats tiller excessively but produce very few heads. The tillers are twisted and thickened and the inside tissue browns. Rot develops and many tillers die. Of 130 varieties of oats tested for resistance in the field in cooperation with T. R. Stanton, Division of Cereal Crops and Diseases, U. S. Department of Agriculture, only 3 varieties (Victoria, Capa, and Pampa) showed no evidence of the disease; 68 per cent showed low resistance, 18 per cent medium resistance, and 14 per cent good resistance.

The Use of Carbon Bisulphide in the Control of Armillaria Root Rot. HAROLD E. THOMAS and LEWIS O. LAWYER. The results of 3 years' work to determine the effectiveness of carbon bisulphide in eradicating the fungus *Armillaria mellea* from orchard soils, indicate low soil moisture to be the most important single factor. Soil textures ranging from light sandy loams to moderately heavy clay loams appeared to influence but little the effectiveness of the carbon bisulphide. Kill of the fungus to a depth of 60 inches is obtained under a wide range of conditions when 45 cc. of the material is applied 8 to 9 in.

deep at 18-inch staggered intervals. With low soil moisture and a surface blanket of moistened earth 3 to 6 in. in depth, complete kill to 70 and 80 in. has occurred. Doubling the rate of application did not materially affect the depth of kill. The death of the fungus in the tree roots is extremely slow in comparison to the rapid diffusion of carbon bisulphide. Where effectively applied, 30 to 60 days is required before the fungus is completely killed in all roots. Eradication of the fungus from orchard soils seems possible if application is made under ideal conditions.

Root Rot of Ranunculus asiaticus Caused by Pythium debaryanum. C. M. TOMPKINS and J. T. MIDDLETON. A disease of the Persian buttercup (*Ranunculus asiaticus*) was observed in some commercial plantings at Inglewood and Pacific Palisades, California, in the winter of 1938 and in similar plantings in the winter of 1939 at Santa Cruz and San Francisco. The most striking symptoms of the disease consist of a general wilting, followed by collapse and death of the plant. The roots, tubers, stems, and petioles of the plant are affected. The roots and tubers of infected plants are dark-brown, water-soaked, and flaccid. The stem plate is discolored, dark, usually not water-soaked, and not flaccid. Occasionally, the petioles become infected. Diseased petioles are dark brown, frequently exhibiting necrotic streaks about 1 to 3 mm. wide and about 30 to 60 mm. long, extending outward from the base of the petiole and parallel to its axis. Isolations made from diseased material have consistently yielded a fungus that has been identified as *Pythium debaryanum.* The organism proved pathogenic when introduced into autoclaved soil. Artificially inoculated plants exhibited symptoms identical with those of naturally infected plants. Though *P. ultimum* and *P. irregulare* are commonly associated with root rots of various herbaceous ornamentals in California, they have not been found associated with this disease. Inoculation experiments have shown that *P. ultimum* is strongly pathogenic to *Ranunculus. P. irregulare* is weakly pathogenic but is likewise capable of inducing disease. Symptoms produced by these two species are similar to those produced by *P. debaryanum.*

Evidence of Production of Antibody-like Substances in Turkish Tobacco, Nicotiana tabacum, Infected with Curly-Top Virus. J. M. WALLACE. That *Nicotiana tabacum* plants recovered from curly top contained virus of unchanged virulence was shown by transfer of the virus to healthy plants by means of the insect vector. Grafting cions from recovered plants on healthy plants usually resulted in mild symptoms. Severity of symptoms varied to some extent and was influenced by conditions of experiments. If the cion was grafted on the side of the healthy plant the terminal shoot of the latter developed mild symptoms. However, if the top of the healthy plant was removed at time of grafting, new axillary shoots frequently showed conspicuous symptoms, at first, then milder symptoms, and, finally, almost normal appearance. Tobacco plants infected by grafting with recovered plants showed the same resistance to reinoculation as plants recovered from severe curly top resulting from inoculation by leaf hoppers. The apparent *passive immunization* of plants suggests the presence of protective substances in the plants showing the acquired tolerance.

The Effect of Petroleum Oil Emulsion on the Fungicidal Value of Bordeaux Mixture. E. E. WILSON. Emulsified, dormant type petroleum oil increased adhesiveness of Bordeaux when used at the rate of 3 to 4 gal. per 100 gal. of the fungicide. One gal. of oil per 100, however, did not increase adhesiveness. In 1936–37 marked improvement in control of peach blight (*Coryneum beijerinckii*) was apparently attributable to the increased adhesiveness. In 1937–38 the improved control incident to the use of oil-Bordeaux, though noticeable, was not so striking, inasmuch as Bordeaux, without oil, gave relatively efficient protection throughout the season. Laboratory washing tests showed that oil retarded the loss of calcium and sulphate from dried Bordeaux films. The wash water from oil-Bordeaux films, however, contained more soluble copper than wash water from Bordeaux films. Apparently the oil dissolved some of the copper from the Bordeaux, and, escaping during the washing process, the oil carried this copper with it. The amount of copper lost in this way was not enough to counterbalance the benefits arising from the increase in adhesiveness imparted by the oil. The effect of this oil-dissolved copper on the toxicity of the wash water to spores was not conclusively determined. Dried films, made from successively diluted Bordeaux and oil Bordeaux, were tested for toxicity to spores of *Coryneum beijerinckii* and *Sclerotinia fructicola.* At the greater dilutions oil-Bordeaux appeared somewhat less toxic than Bordeaux.

Attempts at the in vitro Culture of Erysiphe polygoni and Peronospora destructor. C. E. YARWOOD. The effect of various physical and chemical treatments on the germination and growth of conidia of *Erysiphe polygoni* from bean and clover, and of the sporangia of *Peronospora destructor* from onion was tested on glass slides and on plain agar. Treatments of *E. polygoni* found to increase the percentage germination or length of germ tubes or both under given conditions were: heating the conidia *in situ*, before their use in germination tests, drying the conidia on glass slides, exposure of the conidia to light during germination, placing host leaves in the germination chamber, and addition of potassium permanganate, glutathione, tryptophane, sucrose, extracts from host leaves, and extracts from young cultures of *Thielaviopsis basicola* to the culture medium. The stimulus of host leaves to spore germination was destroyed by heating the leaves. Substances that increased the growth on agar of *P. destructor* were: potassium permanganate, glycine, cysteine hydrochloride, extracts from host leaves, and extracts from young cultures of *Phytophthora citrophthora*. The maximum growth on agar media of mycelium from a single conidium of *E. polygoni* was 400 μ and from a single sporangium of *P. destructor* was 3900 μ.

Stamen Blight of Blackberry Caused by Hapalosphaeria deformans. S. M. ZELLER. This disease infects the winter buds during March. The fungus evidently is not systemic. The winter buds are rather open, the fungus probably entering between the leaf-like scales and infecting the anthers superficially within the calyx. All winter buds are completely emasculated. Long before the flower buds open a fungous pseudoparenchyma entirely surrounds the pollen locule and parasitizes the pollen grains. Pycnidia produced from the surface of the pseudoparenchyma become erumpent and emit coils of spores from the surface of the anther. Emasculated flowers may produce deformed berries by bee pollination. Boysenberry, Youngberry, and *Rubus laciniatus* and *R. macropetalus* have been found infected in Oregon. Previously, the disease has been known from British Columbia and Europe. Bordeaux mixture, applied in January, February, and March, gave no apparent control.

NOTICE TO THE MEMBERSHIP

STANDING RULES ADOPTED BY THE AMERICAN PHYTOPATHOLOGICAL SOCIETY FOR PAPERS AND ABSTRACTS FOR THE COLUMBUS MEETING

3. Members who plan to present papers at the annual meeting must submit to the Secretary three copies of each abstract. These abstracts must be clear and concise, contain no tabular data, and not exceed 200 words in length. They should include only statements of fact, unpublished information, and directly derived conclusions or hypotheses. Reports of progress, or of disease occurrences, or of routine tests of ordinary control measures, are not desired, unless new and significant developments are clearly indicated.

a. *Date Due.* Abstracts must be received by the Secretary on or before November 1st. Members are requested not to submit abstracts unless they expect to attend the meeting.

b. *Number of.* Each member is limited to two papers on which he may appear as sole, senior or junior author.

c. *Editing and Reviewing of.* Abstracts are to be reviewed by a committee appointed annually by the Editor-in-Chief of PHYTOPATHOLOGY and this committee is directed to return to authors for revision such abstracts as fail to meet the above requirements.

d. *Time Limit on Papers.* Members are requested to limit presentation time to 5 to 10 minutes. The maximum time allowed for other than invitation papers will be 15 minutes. Complicated tables or graphs should not be shown.

COMPARATIVE STUDIES OF THE BACTERIA ASSOCIATED WITH POTATO BLACKLEG AND SEED-PIECE DECAY[1]

REINER BONDE[2]

(Accepted for publication April 28, 1939)

INTRODUCTION

In the course of the writer's investigation of the factors involved in potato seed-piece decay and potato blackleg (2, 3, and 4), a relatively large number of cultures of bacteria were isolated from various sources. In order to learn the significance of the various bacteria encountered, it was necessary to know not only the extent of their pathogenicity, but also their identity, in so far as possible. This paper is a record of a comparative study of 62 isolates including authentic cultures of *Bacillus phytophthorus* Appel and *Bacillus carotovorus* Jones (*Erwinia carotovora* (Jones) S. A. B.).

PREVIOUS COMPARISONS OF THE SOFT-ROT AND BLACKLEG PATHOGENS

The identification of the blackleg pathogen and the soft-rot bacteria has long been a problem and has been studied by numerous workers. The literature pertaining to the diseases caused by these pathogens has been summarized by Harding and Morse (8) and by Leach (10 and 12).

The early workers on blackleg and soft rot described, for each of the two, numerous bacteria as the causal organisms and generally considered these diseases as being distinct from each other. The marked similarity of these bacteria, however, resulted in so much confusion that it was not until quite recently that it was suggested that they might be identical.

Lacey (9) was the first to report isolation of *Bacillus carotovorus* from a naturally infected potato. Leach (10, 12) made a comparative study of many strains of pathogenic bacteria isolated from the various stages of the life cycle of the seed-corn maggot, from the soil, from many different vegetables, and from potato affected with blackleg, and included authentic cultures of *B. phytophthorus* and *B. carotovorus.* He attempted to distinguish *B. carotovorus* from the blackleg pathogen on the basis of morphological, physiological, and pathological characteristics, but was unable to do so. He found slight variations in minor characters among the cultures isolated. His conclusion was (12) "that blackleg is nothing more than soft rot of potato and that the bacteria previously designated as *B. phytophthorus* Appel, *B. atrosepticus* van Hall, *B. solanisaprus* Harrison, and *B. melanogenes* Peth. and Murph. are merely strains of the earlier described species, *B. carotovorus* Jones."

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[1] Part of a dissertation submitted to the Graduate School of the University of Minnesota in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Plant Pathology.

[2] The writer is indebted to Dr. J. G. Leach and Dr. E. C. Stakman for their advice and criticism during the progress of this work and to Dr. Donald Folsom for suggetions pertaining to the preparation of the manuscript.

This concept presented by Leach greatly simplifies the problem of the etiology of this large group of plant diseases that includes the soft rots of numerous fleshy vegetables and plants as well as the much debated potato blackleg. This is especially true in view of the great amount of contradictory literature that already has appeared pertaining to the identity of the organisms responsible for this group of plant diseases. According to Leach (10) the synonymy of the blackleg pathogen is as follows:

Bacillus carotovorus Jones. Centralb. f. Bakt. 7: 12. 1901.
Bacillus atrosepticus van Hall. Inaug. Diss. Univ. Amsterdam. 1902.
Erwinia atroseptica (van Hall). Comm. S.A.B. Man. Determinative Bact. 1923.
Bacillus phytophthorus Appel. Ber. d. deut. bot. Ges. 20: 128. 1902.
Erwinia phytophthora (Appel). Comm. S.A.B. Jour. Bact. 5: 191. 1920.
Bacillus solanisaprus (Harrison). Centralb. f. Bakt. II, 17: 24. 1907.
Erwinia solanisaprus (Harrison). Comm. S.A.B. Jour. Bact. 5: 191. 1920.
Bacillus melanogenes Pethybridge and Murphy. Proc. Roy. Irish Acad. 29B: 1. 1911.
Bacillus oleraceae (Harrison). Science (n. s.) 16: 152. 1902.
Bacillus omnivorus van Hall. Inaug. Diss. Univ. Amsterdam. 1902.
Bacillus apivorus Wormald. Jour. Agr. Sci. 6: 203–219. 1914.
Erwinia oleraceae (Harrison). Comm. S.A.B. Man. Determinative Bact. 1923.

The genus *Bacillus,* however, according to the London Congress 1936 (15), is invalid for bacterial species not producing endospores and, therefore, cannot be used for naming non-spore-forming plant pathogens. Because of this decision and the fact that the generic name *Erwinia* is now quite generally adopted to designate plant pathogenic rods with peritrichiate flagella, it would seem that *Erwinia carotovora* (Jones) S.A.B. is the proper name for the blackleg and soft-rot pathogen described by Leach.

SOURCES OF ISOLATES

The sources of the bacterial cultures used in these comparative studies and the types of disease which they cause on the potato are recorded in table 1. The pathogens were secured from a rather wide range of hosts and environmental conditions. Many were found closely associated with certain insects. Cultures SC[3] 1 to 4 were secured from the surface of eggs of the seed-corn maggot, *Hylemyia cilicrura* Rondani, which had been deposited in sterilized soil under controlled conditions near Charleston, South Carolina. Culture M[3] 31 was obtained from the surface of eggs of the cabbage maggot, *H. brassicae* (Bouché), found in the field near Presque Isle, Maine.

Cultures SC 5, 6, and SC 15 to 22 were isolated from the burrows of the seed-corn maggot found infesting potato seed pieces under field conditions. This group of cultures was obtained from potato seed pieces secured from the truck-garden areas in the vicinity of Charleston, S. C. Culture M 9 was secured from similar material from a potato field in northern Maine.

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[3] SC refers to cultures isolated in South Carolina; M refers to cultures isolated in Maine.

In certain experiments potato seed pieces were inoculated with pathogenic bacteria by means of the larvae of the seed-corn maggot under controlled laboratory conditions. Cultures SC 7 to 11 were secured from infections induced in this way.

Several cultures were isolated from within the puparia of Hylemyid insects. Culture M 18 was obtained from a puparium of the seed-corn maggot reared in the laboratory in connection with certain insect inoculation studies. Two other cultures (M 10 and 11) were isolated from the inside of a puparium of the cabbage maggot, which had hibernated in the soil under field conditions. These latter cultures are especially interesting because they have shown that certain soft-rot bacteria may be harbored throughout the severe winter of northern Maine, within the resting stage of a common field insect.

Other cultures were secured from decaying plants infested with these Hylemyids. Isolates SC 13 and 24 were taken from a decayed spinach plant and a decayed bean seed, respectively, that were infested with the seed-corn maggot. Isolates M 19 to 22 were isolated from decaying plants belonging to different members of the Cruciferae that had been attacked by the cabbage maggot.

During these studies attempts were made to determine whether other insects besides the Hylemyids might not also be vectors of soft rot and other bacterial diseases of the potato. A certain Staphylinid insect has been found to be very commonly associated with decayed plant material and with the blackleg disease. Cultures M 12 and 13 were isolated from potato seed pieces infested with this insect. Another organism (culture C), pathogenic to the potato, was found present in a carrot infested with the larvae of the carrot rust fly, *Psila rosae* Fabricius.

The writer has spent considerable time investigating sources of infective material responsible for seed-piece decay and blackleg. Previous studies (2) had indicated that the initial source of infection often did not originate in the seed stock, as is commonly accepted to be true by research workers and by growers. However, culture M 8 was capable of causing blackleg, and was secured from a seed potato tuber having late-blight dry rot. The bacterial soft-rot organism apparently had entered secondarily following late-blight decay. Also, 3 pathogenic cultures (M 14, 27, and 32) were isolated from the lesions of potato scab caused by the fungus, *Actinomyces scabies* Thaxter.

The soil contained bacteria pathogenic to the potato. Three cultures (SC 12, 23, and 25) were isolated from the soil in the Charleston truck-crop area. These cultures were secured by the host-selection method employed by Leach (11). Culture SC 26 is especially interesting because it originated as a subculture from SC 25, but differs from it by being nonchromogenic on

TABLE 1.—*Pathogenic cultures: summary of sources of isolates and types of pathogenicity on potato*

Isolate No.[a]	Location	Source of isolates	Pathogenicity to potato	
			Seed pieces	Stems
SC 1 to 4	Charleston, South Carolina	Surface of eggs of *H. cilicrura* deposited in sterilized soil (laboratory studies)	White decay	Blackleg
SC 5 and 6	Do.	Potato seed piece infested with young larvae of *H. cilicrura* (field infestation)	Do.	Do.
SC 7 to 11	Do.	Potato seed pieces inoculated with *H. cilicrura* larvae in sterilized soil (laboratory studies)	Do.	Do.
SC 12	Do.	Potato soil (isolated by Leach method)	Do.	Do.
SC 13	Do.	Spinach infested with *H. cilicrura* (field infestation)	Do.	Do.
SC 14	Do.	Potato blackleg plant	Do.	Do.
SC 15 to 22	Do.	Potato seed piece infested with young larvae of *H. cilicrura* (field infestation)	Yellow and cream-colored decay	Trace in pith
SC 23	Do.	Potato soil (isolated by Leach method)	Yellow decay	Trace in pith
SC 24	Do.	Decayed bean. Inoculated with *H. cilicrura*	Do.	Do.
SC 25	Charleston, South Carolina	Potato soil (isolated by Leach method)	Yellow decay	Vascular infection
SC 26	Do.	Originated as variant subculture from SC 25	White decay	Do.
M 1 to 4	Aroostook County, Maine	Blackleg plant	Do.	Blackleg
M 5	Do.	Small potato plant with necrotic base or foot. Type of disease questioned	Do.	Do.
M 6 and 7	Fort Fairfield, Maine	Tubers infected also with the bacterial wilt and soft rot recently discovered in Maine	Do.	Slight necrosis in pith
M 8	Presque Isle, Maine	Tuber affected with late blight	Do.	Blackleg
M 9	Do.	Potato seed piece infested with larvae of *H. cilicrura* (field infestation)	Do.	Do.

[a] SC refers to cultures isolated in South Carolina; M refers to cultures isolated from Maine material.

TABLE 1.—(*Continued*)

Isolate No.[a]	Location	Source of isolates	Pathogenicity to potato	
			Seed pieces	Stems
M 10 and 11	Do.	Puparium of *H. brassicae.* Overwintered in field	Do.	Do.
M 12 and 13	Do.	Potato seed piece infested with Staphylinid insect (field infestation)	Do.	Do.
M 14	Do.	Scab lesions on potato tuber	Do.	Do.
M 15 and 16	Do.	Discolored vascular bundles of Irish Cobbler tuber	Do.	Trace in pith
M 17	Do.	Stem end browning and discolored vascular bundles in Irish Cobbler tuber	Slow whitish-brown decay	Do.
M 18	Presque Isle, Maine	Puparium of *H. cilicrura* (reared in laboratory experiments)	Slow whitish-brown decay	Trace in pith
M 19	Do.	Cabbage associated with larvae of *H. brassicae*	Do.	Do.
M 20	Do.	Turnip infested with larvae of *H. brassicae*	Do.	Do.
M 21	Do.	Cabbage infested with larvae of *H. brassicae*	Do.	Do.
M 22	Do.	Decayed Chinese cabbage associated with larvae of *H. brassicae*	Do.	Do.
M 23 to 26	Do.	Surface lesions on potato seed pieces from soil infection	Do.	Do.
M 27	Do.	Scab lesions on potato tuber	Do.	Do.
M 28 to 30	Do.	Discolored vascular bundles of Irish Cobbler potato	Do.	Do.
M 31	Do.	Eggs of *H. brassicae* (field)	Yellow decay	Do.
M 32	Do.	Scab lesions on potato tuber	Do.	Do.
A	Germany	*B. phytophthorus* furnished by J. G. Leach	White to brown decay	Blackleg
L 1 and 2	Minnesota	*B. carotovorus* furnished by J. G. Leach	Do.	Do.
C	Presque Isle, Maine	Carrot infested with carrot rust fly, *Psila rosae* Fabricius	White decay	Trace in pith

potato slices and other media. It possesses, however, all of the other characters of the original parent culture.

Four other cultures (M 23 to 26) were isolated by a different method from soil in a potato field in Aroostook County, Maine. Freshly-cut potato seed pieces were planted in the field under rather moist conditions. After several days, shallow lesions had formed on the cut surfaces of these seed pieces. The tissue beneath the lesions was transferred to agar in Petri dishes, and the cultures thus isolated were later tested for pathogenicity prior to being purified and subjected to the tests necessary for determining their differential characters.

It is deemed possible by some workers that the organism responsible for potato blackleg may remain dormant within the seed tubers from year to year, and produce disease symptoms only when the environmental conditions are favorable. Although this may be possible, the writer has never isolated the organism from the tubers of apparently healthy plants. Further, from tubers having discolored vascular rings, certain bacteria have been secured that are pathogenic to potato slices, but the organisms isolated from this source have been incapable of producing blackleg when inoculated into potato stems.

Physiological characteristics indicate that some of the isolates (M 28 to 30) secured from discolored vascular bundles of potatoes belong to a somewhat different group of bacteria. Cultures M 15, 16, and 28 to 30 were isolated from the diseased vascular bundles of potato tubers. Culture M 17, quite similar to M 28, is of special interest because it was isolated from a tuber having the diseased condition known as "stem end browning."

Perhaps more cultures isolated from tubers and potato plants affected with the blackleg disease should have been used in this comparison. Five cultures (M 1 to 5), however, were secured from diseased potato plants from widely distributed fields in Aroostook County for the purpose of determining the variations that exist between the different strains of the blackleg pathogen. Culture M 5 was obtained from a blackleg plant with rather atypical symptoms of the disease. Such plants generally occur early in the season and are characterized by being small (4 to 6 inches high) and by having decayed stem bases, resembling somewhat the symptoms caused by an excessive amount of water. This isolate was included to determine whether this diseased condition is caused by the blackleg organism. Culture SC 14 is the only one studied that originated from a blackleg plant in Charleston, South Carolina. Culture A, secured from Germany through Leach, was designated as *Bacillus phytophthorus* Appel and was considered to be an authentic blackleg pathogen. Cultures L 1 and L 2 are considered to be authentic strains of *B. carotovorus* both of which were obtained from J. G. Leach.

Cultures M 6 and M 7 were obtained from potato tubers affected with bacterial wilt and the bacterial soft rot recently discovered in Maine, apparently present as secondary invaders.

LABORATORY METHODS

The procedures used in the pure-culture studies were those recommended by the Society of American Bacteriologists (6), except where specific deviations are mentioned.

The pathogenic organisms were isolated in pure form by the commonly accepted bacteriological methods. Cultures were maintained on potato dextrose agar and nutrient broth until needed for the various tests. The different cultures of bacteria were replated and single-colony cultures isolated prior to each series of tests. By this method inconsistent reactions caused by contamination were largely eliminated. The purified cultures, all originating from single-colony isolations, were also tested for pathogenicity on potato slices just before being subjected to the differential tests. No determination was based on a single test. The cultures for each comparison were made in duplicate or in triplicate and the comparisons were repeated until conclusive results were obtained.

PATHOGENICITY

The pathogenicity of the different cultures on the potato is summarized in table 1. A study of the data reveals that these pathogens cause several distinctly different types of disease symptoms.

Certain cultures included in these studies produced a very rapid, white decay on potato slices and also caused blackleg when introduced into the stems of young potato plants. These included strains SC 1 to 14, which were of southern origin. The strains M 1 to 5 and M 8 to 14, having very similar pathogenic qualities, were isolated from the potato-growing areas of Aroostook County. Cultures designated as A and L 1 and 2, also belong to this group. Strains designated C and M 6, 7, 15, and 16 are quite similar in pathogenicity. They are, however, only weakly pathogenic on potato stems.

Although bacteria capable of causing a rapid, white decay of potato tubers and blackleg on potato plants were commonly encountered, other bacteria that are less easy to describe and identify were recovered. Included in this group are cultures SC 15 to 22. Bacteria of this kind were commonly isolated in South Carolina from decaying potato seed pieces. A similar organism was also isolated from the puparia of the seed-corn maggot but was not studied in detail. The organisms of this group are less virulent on potato slices than the soft-rot bacteria previously referred to, and have not produced blackleg symptoms when inoculated into potato stems. These bacteria cause a rather slowly developing, yellow or cream-color decay on potato slices. Culture SC 23 possibly belongs to the same group. The latter culture, however, differs from those previously referred to by its ability to peptonize litmus milk.

Bacterial strain SC 25 produces very similar pathological symptoms on potato slices but differs in certain fundamental morphological characteristics. Also, it, as well as its nonchromogenic subculture SC 26, appears to be a vascular parasite. Cultures M 31 and 32 also cause a yellow decay of potato slices and appear to be somewhat similar to SC 25 as to pathogenicity.

TABLE 2.—*Pathogenic cultures: summary of morphological and physiological characteristics*[a]

Isolate No.	Morphology					Oxygen			Nitrates		Carbohydrates										Milk				
												Dextrose			Lactose			Sucrose							
	Short rods	Flagella	Chains	Capsules	Gram	Aerobe	Facultative anaerobe	Gelatin liquefaction	Reduction	Gas	Diastatic action	Acid	Alkaline	Gas	Acid	Alkaline	Gas	Acid	Alkaline	Gas	Curd	Peptonization	Gas	Indole	Hydrogen sulphide
SC 1	+	Pr		–	–	+	+	+	+	–	–	+		–	+			+		–	+	–	–	+	+
SC 2 to 5	+	Pr		–	–	+	+	+	+	–	–	+		–	+			+		–	+	–	–	–	+
SC 6	+	Pr	+	–	–	–	+	+	+	–	–	+		+	+		+	+		+	+	–	–	–	+
SC 7 and 8	+	Pr		–	–	+	+	+	+	–	–	+		–	+			+		–	+	–	–	+	+
SC 9	+	Pr	+	–	–	+	+	+	+	–	–	+		–	+			+		–	+	–	–	+	–
SC 10 to 12	+	Pr		–	–	+	+	+	+	–	–	+		–	+			+		–	+	–	–	+	+
SC 13	+	Pr	–	–	–	+	+	+	+	–	–	+		+	+		+	+		+	+	–	–	–	U
SC 14	+	Pr	+	–	–	+	+	+	+	–	–	+		+	+		+	+		+	+	–	–	–	–
SC 15	+	Pl	+	–	–	–	–	+	+	–	+		+	–		+	–		+	–	–	–	–	–	–
SC 16 to 22	+	Pl		–	–	–	–	+	+	–	+		+	–		+	–		+	–	–	–	–	–	–
SC 23	+	Pl		–	–	+	–	+	+	–	+		+	–		+	–		+	–	–	+	–	–	–
SC 24	+	Pr	+	–	–	+	–	+	+	+	+		+	–		+	–		+	–	–	+	–	–	U
SC 25 and 26	+	Pr		–	–	+	+	+	+	+	+		+	–		+			+	–	–	+	–	–	+
M 1 to 5	+	Pr		–	–	+	+	+	+	–	–	+		–	+		+	+		+	+	–	–	U	U
M 6	+	Pr		–	–	+	+	+	+	–	–	+		–	+		–	+		–	+	–	–	U	U
M 7	+	Pr		–	–	+	+	+	+	–	–	+		+	+		+	+		+	+	–	+	U	U
M 8	+	Pr	+	–	–	+	+	+	+	–	–	+		–	+		–	+		–	+	–	–	–	+
M 9	+	Pr		–	–	+	+	+	+	–	–	+		–	+		–	+		–	+	–	–	–	+
M 10 and 11	+	Pr		–	–	+	+	+	+	–	–	+		–	+		–	+		–	+	–	–	+	–

[a] SC refers to cultures isolated in South Carolina; M refers to cultures isolated from Maine material; Pr = peritrichic arrangement of flagella; Pl = polar arrangement of flagella; T = the determinations of these characteristics were somewhat doubtful and are tentative only; S = positive in slight amount; U = undetermined.

TABLE 2.—(*Continued*)

Isolate No.	Morphology				Gram	Oxygen		Gelatin liquefaction	Nitrates		Carbohydrates										Milk			Indole	Hydrogen sulphide
												Dextrose			Lactose			Sucrose							
	Short rods	Flagella	Chains	Capsules		Aerobe	Facultative anaerobe		Reduction	Gas	Diastatic action	Acid	Alkaline	Gas	Acid	Alkaline	Gas	Acid	Alkaline	Gas	Curd	Peptonization	Gas		
M 12 and 13	+	Pr	+	−	−	+	+	+	+	−	−	+		−	+		−	+		−	+	−	−	−	−
M 14 and 15	+	PrT		−	−	−	+	+	+	−	−	+		+	+		+	+		+	U	U	U	−	U
M 16	+	PrT		−	−	+	+	+	+	−	U	+		+	+		+	+		+	U	U	U	−	U
M 17	+	PrT		−	−	+	+	+	+	−	+T	±S		−	±S		−	±		−	U	U	U	U	U
M 18	+	PrT		−	−	−	+	+	+	−	+	±		−	−		−			−	−	+	−	−	−
M 19	+	PrT		−	−	+	+	+	−	−	+	−		−	−		−			−	−	+	−	−	−
M 20	+	PrT		−	−	+T	−T	+	−	−	+	−		−	−		−			−	−	−	−	−	−
M 21	+	PrT		−	−	+T	−T	+	+	−	+	−		−	−		−			−	−	−	−	−	−
M 22	+	PrT		−	−	+	−	+	+	−	+	±		−	−		−	−		−	−	+	−		−
M 23	+	PrT		−	−	+	−	+	+	−	+	−		−	±		−	±		−	−	+	−	−	−
M 24	+	PrT		−	−	+	−	+	+	−	+	−		−	−		−	−		−	−	+	−	−	+
M 25	+	PrT		−	−	+	−	+	−	−	+	±		−	−		−	−		−	−	+	−	−	−
M 26	+	PrT		−	−	+	−	+	+	−	+	−		−	−		−	−		−	−	+	−	U	U
M 27	+	PrT		−	−	+	−	+	+	−	+	±		−	−		−	−		−	−	+	−	−	U
M 28	+	PrT		−	−	+	−	+	+	−	+	−		−	−		−	−		−	−	+	−	−	U
M 29	+	PrT		−	−	+	−	+	+	−	U	+		−	−		−	−		−	U	U	U	−	U
M 30	+	PrT		−	−	+	−	+	+	−	U	−		−	−		−	−		−	U	U	U	−	+
M 31	+	PrT	+	−	−	+	+	+	+	−	+	−		−	−		−	−		−	−	+	−	+	−
M 32	+	PrT	+	−	−	+T	−	+	+	−	+	+		−	−		−	−		−	−	−	−	−	U
A	+	Pr	+	−	−	+	+	+	+	−	−	+	−	+	+	−	+	+	−	+	+	−	−	+	+
L 1 and 2	+	Pr		−	−	+	+	+	+	−	−	+	−	−	+	−	−	+	−	−	+	−	−	U	U
C	+	Pr	+	−	−	+	+	+	+	−	−	+	−	+	+	−	+	+	−	+	+	−	+	−	−

Isolations made in Aroostook County have very often yielded weakly parasitic bacteria of a distinct type. These bacteria appear to be very generally distributed and have been isolated from a variety of sources. They have been secured from plant material including the potato and the different members of the Cruciferae. It appears that these organisms may be responsible for much of the bacterial decay incident in Chinese cabbage, common cabbage, cauliflower, and turnip. These organisms have been taken also from within and puparia of the seed-corn maggot and the cabbage maggot, as well as from the soil. They produce a slow, white to brownish decay on potato seed pieces, but do not cause blackleg. Among this group are included cultures M 18 to 26, all isolated in Maine. A very similar organism represented by cultures M 28 to 30 has been isolated from the vascular bundles of potato tubers.

Recently a bacterium has been found present in tubers afflicted with "stem-end browning." This organism has been obtained from tubers grown in Maine. Represented by M 17, it causes a rather slow, white to brown decay on potato slices, and may possibly be similar to those organisms obtained from the vascular bundles of potato tubers referred to previously.

It should be observed that culture M 5, which was isolated from a small plant with a stem darkened at the base, as described previously, yielded the blackleg organism. Growers and inspectors alike have been confused regarding the diagnosis of this diseased condition. The soft-rot or blackleg bacterium was isolated fairly consistently from this type of plant, which would indicate presence of blackleg. When blackleg occurs early in the season, many of the diseased plants are small and the symptoms are not typical. If the disease appears later in the season, when the plants are larger, more typically backleg plants result.

MORPHOLOGY

The morphological and physiological characteristics of the various bacterial cultures are summarized with others in table 2.

Relative Size

It may be observed from the data recorded in table 2 that all of the organisms are, relatively, short rods. The cells of the different bacterial strains varied in length from 1.5 to 3.0 μ and in width from 0.6 to 0.7 μ. Although the different strains varied somewhat in length, the degree of difference in this respect was not sufficient for a practical differentiation.[4] It is generally recognized that such variations are to be expected among bacterial cultures. Morse (13), in his studies with the different organisms causing blackleg, found that closely related species or different strains of the same species differed in their mean dimensions.

None of the bacteria studied formed spores or capsules. The cells were, for the most part, either single or in pairs. An occasional chain was recorded from the following strains: SC 6, 9, 13, 14, 15, and 24, M 8, 12, 13, 31, and 32, A, and C. This variation is considered of insufficient importance for use as a differential characteristic.

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[4] The organisms were grown on nutrient broth and stained with Ziehl's carbol-fuchsin prior to microscopic observation and measurement under an oil-immersion objective.

Arrangement of Flagella

All of the bacteria were actively motile. It was found that they belonged to 2 different groups when classified on the basis of the arrangement of the flagella.[5] It may be noted in table 2 that some of the organisms had from 3 to 6 rather long polar flagella. This group of organisms was further characterized by ability to produce a yellow or cream-color, rather slowly developing decay on potato slices. They also produced a yellow pigment when grown on various kinds of media. Cultures SC 15 to 23 are among the isolates belonging to this group. These cultures, with the possible exception of SC 23, appeared to be identical one with another in other differential characteristics. The writer has been unable to find reference to this organism in the literature.

All the other bacteria, which were examined in detail, possessed peritrichic flagella. It may be observed that the arrangement of the flagella of some cultures listed in table 2 was not definitely determined. Observations of inconclusively stained material indicated that the flagella of these cultures probably also had a peritrichic arrangement.

PHYSIOLOGICAL CHARACTERISTICS

The physiological characteristics of the isolates referred to in these studies are recorded in table 2. The bacterial strains proved in all cases to be Gram-negative. The Hucker modified method, as outlined by the Committee of the Society of American Bacteriologists, was followed. Each determination included a Gram-positive and a Gram-negative organism, one on either end of a slide, with the unknown between them.

Relation to Free Oxygen

The relation of the different organisms to oxygen was determined. The determination was based on the amount of growth in the closed arms of fermentation tubes and by observing the place of maximum growth in agar shake tubes and in inverted Durham tubes in nutrient broth containing bromcresol purple indicator.

The blackleg or soft-rot bacteria were all aerobic and facultative anaerobes. Included in this group were the following cultures: SC 1 to 14, M 1 to 16, A, L 1 and 2, and C. (Cultures M 6, 7, 15, and 16, and C are included in the soft-rot group because of their morphological and physiological characters, although they are only weakly pathogenic on potato stems.) Cultures SC 25 and 26, and M 17, 18, 19, and 31, were also facultative anaerobes, but do not belong to the blackleg or soft-rot group. The remaining cultures were apparently all rather strict aerobes. It was noted that these strict aerobes were for the most part more weakly pathogenic on the potato than were the facultative anaerobes that caused rapid soft rot.

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[5] Loeffler's, Shunk's and Gray's methods for staining flagella were employed according to the procedure outlined in the Manual of Methods for Pure Culture Study of Bacteria issued by the S.A.B. for 1930.

Liquefaction of Gelatin

Liquefaction of gelatin was determined by means of stab cultures on nutrient gelatin incubated at 20° C. The cultures all caused a rapid liquefaction of this medium. All of the bacteria causing blackleg and soft rot, including those cultures previously described in this discussion as being aerobic and facultative anaerobes, produced a rapid infundibuliform and sometimes crater-form type of liquefaction. Complete liquefaction by these cultures required approximately from 8 to 10 days. The aerobic cultures all produced a stratiform type of liquefaction, which was somewhat slower than that of the blackleg or soft-rot strains.

It can thus be seen that the type of liquefaction produced was another means by which the pathogens could be separated into 2 distinctly different groups. Those producing a rapid, infundibuliform type of liquefaction on gelatin were in most cases causes of blackleg and soft rot. On the other hand, those producing a stratiform type of liquefaction were generally strict aerobes and incapable of producing blackleg. Cultures of this type were in most cases rather slow producers of decay on the potato.

Reduction of Nitrates

The reduction of nitrates was detected by the sulphanilic acid-∞-naphthylamine test. Only 3 of the bacterial strains (M 19, 20 and 25) failed to reduce nitrates to nitrites in these comparisons. Most of the cultures caused this reaction quite rapidly and produced a dark red discoloration within 2 days in the broth cultures when the necessary reagents were added. However, certain cultures (SC 15 to 23 and M 21 and 22) gave a less distinctly positive reaction and produced only a pink discoloration, even after 10 to 15 days' growth on the medium. It should be observed that the cultures that failed to reduce the nitrates or did so weakly were incapable of producing blackleg. These cultures, as a group, were less pathogenic on potato than those that actively reduced the nitrates.

Three of the bacterial strains (SC 24, 25, and 26) produced gas on nitrate medium in addition to their reducing nitrates to nitrites.

Reaction to Carbohydrates

Diastatic Action. The diastatic reaction on different carbohydrates has been considered one of the most satisfactory methods for identifying certain bacterial plant pathogens. Two methods outlined in the Manual of the Society of American Bacteriologists (6) were employed in determining the diastatic action for the different bacteria. By one method the various cultures were grown on Petri dishes of a 0.2 per cent soluble starch-beef extract agar, and tested for starch hydrolysis by flooding with a saturated solution of iodine in 50 per cent alcohol after different periods of growth. Most of the cultures were unable to hydrolyze starch. However, when the Eckford method was used, certain of the bacterial isolates gave positive tests. It is seen from table 2 that cultures SC 15 to 26, M 17 to 28, 31, and 32, were capable of hydrolyzing starch. The diastatic action was not tested for cultures M 16, 29, and 30.

It may be observed from tables 1 and 2 that none of the bacterial strains that hydrolyzed starch in these tests was capable of producing blackleg. Most of these bacteria were less pathogenic on the potato than those of the soft-rot group and also in most cases were responsible for a rather slowly developing yellow or brownish-white decay on potato seed pieces. On the other hand, all of the cultures found capable of causing blackleg were unable to hydrolyze starch.

Fermentation of Sugars and Other Carbohydrates. The fermenting action of the bacteria on the carbohydrates was tested on nutrient broth to which 1 per cent of the respective sugars or other carbohydrates had been added. Durham tubes containing brom-cresol purple indicator in the liquid media were used for the detection of acid and gas. The results of these tests are recorded in table 2.

It is to be observed that all of the blackleg and soft-rot cultures formed acid on dextrose, lactose, and sucrose. Culture M 17, not of this group of bacteria, formed a slight amount of acid on these sugars. In separate tests, not recorded in the table, these cultures also produced acid from mannite and salicin and, in some tests, from glycerin.

The ability to produce gas on the carbohydrates, however, varied considerably with the different cultures. Cultures SC 6, 13, 14, and cultures A, C, and M 7 formed gas from dextrose, lactose, sucrose, and mannite. Cultures M 14, 15, and 16 produced gas on the first 3 sugars, but were not tested on glycerin, mannite, and salicin. Five cultures (M 1 to 5) obtained from blackleg plants in Maine were all alike and produced small amounts of gas on lactose and sucrose but not on dextrose.

It should be observed that many of the blackleg and soft-rot cultures isolated both in South Carolina and Maine failed to form gas on the carbohydrate media. The cultures designated C and M 7 were in all cases very rapid gas formers and produced an abundance of gas on all of the carbohydrate media. It also should be noted that these organisms possess all of the differential characteristics of the blackleg group, but are only weakly parasitic on potato stems.

From these tests it is apparent that the ability to form gas on the different carbohydrate media is not a reliable criterion by which to determine the bacteria responsible for blackleg. This phase of the problem will be discussed in more detail elsewhere in this paper.

An examination of the data in tables 1 and 2 will reveal that the bacterial isolates incapable of producing blackleg have a decidedly different reaction on carbohydrate media than the soft-rot and blackleg group. It is to be seen that most of these cultures did not produce acid or gas on any of the carbohydrate media used. There are, however, a few possible exceptions. Cultures M 18, 22, 25, and 27 produced a slightly acid reaction on dextrose. Culture M 23 produced a slight amount of acid on lactose, sucrose, and glycerin. The production of acid by these cultures was quite slow and appeared only after 5 or more days, and was present only near the top portion of the test tube cultures.

The reactions of the cultures on the different carbohydrate media may serve in differentiating the different groups of bacteria, found to cause certain types of decay, from those causing the blackleg disease. The blackleg bacteria, as a group, produce acid on the different carbohydrate media, and certain strains also may produce gas, while the former group as a rule produces neither acid nor gas.

Another group of pathogenic bacteria does not produce acid and may even cause a slight alkalinity on the different sugars. These bacteria as a group have been less pathogenic, causing a slow decay of potato slices. Certain members of this group also appear to be common pathogens of the Cruciferae and may possibly be the cause of the decay found prevalent in Chinese cabbage, common cabbage, cauliflower, and turnip, and certain other vegetables. Cultures M 19 to 21, 24, 26, 28, and 30 are included in this group for the present.

Reaction on Litmus Milk

Litmus milk was found to be a rather valuable differential medium. It may be observed that the cultures can be divided into several groups on the basis of their action on litmus milk. The blackleg or soft-rot bacteria all produced an acid reaction sufficient in amount to redden the litmus indicator and coagulate milk. None of these isolates, however, peptonized the milk medium, although in some cases a slight amount of whey was produced. The isolates included in this group were the following: SC 1 to 14, M 1 to 5 and 8 to 13, A, and L 1 and 2. The cultures M 6 and 7, and C had a similar reaction, although they did not produce blackleg when inoculated into potato stems. Only 2 cultures, M 7 and C, produced gas on litmus-milk media. It is noteworthy that the latter 2 cultures also produced gas in abundance on the different carbohydrates.

Another group of bacteria does not render the litmus milk acid. Instead, the cultures develop a faint bluish tint. No curd is formed nor is the milk peptonized. Included in this group are cultures SC 15 to 22 isolated from decaying potato seed pieces secured from fields in the vicinity of Charleston, S. C. Certain isolates having these characteristics on litmus milk were isolated also from decaying plant material from Aroostook County, Maine. Cultures M 20 and 21 were secured from decaying turnip and cabbage, respectively, and culture M 32 from a potato-scab lesion.

A third group of pathogens studied by the writer includes all of those bacterial isolates that do not coagulate, but that completely peptonize, litmus milk. There were distinct differences in the color produced by these cultures on this medium. Cultures M 18, 23 to 28, and 31 caused a yellowish coloration of the medium. Culture SC 23 was quite similar in this respect but slightly fluorescent, and cultures SC 25 and 26 caused a distinct orange coloration. Culture SC 24 produced a brownish-gray and cultures M 19 and 22, a bluish green in litmus milk.

Cultures M 14 to 17, 29 to 30, were not grown in litmus milk, so that their reaction to this medium is not known.

Indole Production

The indole test has long been considered one of the least satisfactory of the differential tests employed in pure-culture studies. The writer first tested his isolates for the production of indole by the Ehrlich-Böhme, the Gore, and the Gnezda methods as outlined in the Manual of Methods for Pure Culture of Bactetria (6). The cultures were all negative for indole according to the 3 tests referred to here. A culture known to produce indole gave positive reactions by the Ehrlich-Böhme method, which would indicate that the method was correctly employed.

Following these negative results, secured by the use of the 3 recommended methods, a fourth, the Kovåks method, was employed as outlined by Ruchhoft *et al.* (14). Cultures SC 1, 7 to 12, M 10, 11, 31, and A were found to produce indole when tested by the Kovåks method. It should be observed that all of these cultures, with the exception of M 31, were members of the blackleg or soft-rot group of bacteria. It is of interest that the other members of this group, although capable of producing blackleg and possessing similar cultural characteristics, did not give positive reactions for the production of indole. Certain cultures, M 1 to 7, 17, and 26, and L 1 and 2, were not tested for the production of indole by the Kovåks method.

Production of Hydrogen Sulphide

The production of hydrogen sulphide is one of the differential tests often employed in determining the identity of bacteria. The Committee on the Study of Pure Culture Methods of the Society of American Bacteriologists is not yet ready to recommend any of the methods usually employed for this test.

In the present study the cultures were grown on "Bacto lead acetate agar" (obtained from the Digestive Ferments Co.) on which the production of hydrogen sulphide is indicated by a darkening of the agar.

Cultures SC 1 to 8 and 10 to 12, M 8 and 9, and A, belonging to the blackleg group of bacteria, gave positive tests. Cultures SC 25 and 26 and M 24 and 30 were positive for hydrogen sulphide production, but do not belong to the blackleg or soft-rot group. Cultures SC 13 and 24, M 1 to 7, 14 to 17, 26 to 29, and 32, and L 1 and 2 were not tested on this medium, so their ability to produce hydrogen sulphide is not known. Furthermore, cultures M 24 and 30, belonging to other groups, also produced hydrogen sulphide gas on Bacto lead acetate agar. According to the manual (6) "there are indications, moreover, that the nature of the medium has a considerable influence upon the production of sulphide, and a negative determination must, therefore, be accepted with a good deal of reservation." In view of the results obtained by the writer in these tests and the opinion of the Committee concerning the unreliability of the hydrogen sulphide test, it is felt that it is not reliable enough to be of much service in distinguishing between the groups of cultures in question.

Chromogenesis

The cultures differed in color production when grown on the different media. The cultures producing blackleg all caused a white or slightly cream-color decay on potato slices. The potato slices decayed by these organisms also produced the characteristic black or dark brown discoloration on standing, especially when exposed to dry air. The ability to produce this dark pigment (melanin) varied considerably for the different cultures, and the writer made no attempt to use it in his descriptive characterizations.

Other cultures produced a rich cream or yellow pigment on potato slices and also a yellow pigmentation on nutrient broth. Cultures SC 15 to 25 from South Carolina possessed this characteristic. Cultures M 31 and 32, isolated in northern Maine, also produced quite similar chromogenic reactions. The writer has isolated chromogenic organisms similar to the latter from the soil in the vicinity of Presque Isle, Maine. It is believed that they occur quite commonly and often cause decay of potato seed pieces in the soil.

A bacterium capable of causing a slowly developing, brownish to white decay on potato tubers occurs very commonly in Maine. In liquid medium a light-brown pigment is produced. This organism has been found under a variety of circumstances causing the destruction of fleshy plant material. It has been isolated from the puparia of the seed-corn maggot and also from decaying cabbage, turnip, and Chinese cabbage infested with the cabbage maggot (cultures M 18 to 22). An organism (M 23 and 24) causing a similar decay has been found in characteristic lesions on potato seed pieces planted in the field. This would indicate that it commonly occurs in the soil. Cultures M 25 and 26 also cause a brown decay, but in liquid media a rather dark brown to reddish pigmentation is produced. It would appear that the latter bacteria are somewhat different from the previously mentioned isolates that cause a slightly brownish tint on the medium, but the differences may not be significant.

The data summarized in table 2 show that the blackleg organisms isolated by the writer differ in certain of their physiological characteristics. Blackleg probably is not caused by a specific bacterium but by several closely related organisms that differ from each other in certain minor respects.

COMPARISON OF THE SOFT-ROT OR BLACKLEG BACTERIA ISOLATED IN THESE STUDIES

Examination of tables 1 and 2 will discose the fact that the various soft-rot and blackleg bacteria isolated by the writer answer quite well the description of *Bacillus carotovorus* given by Leach (10). In some tests, however, they produced acid on glycerin medium, which is contrary to the results reported by Leach. In the tests with carbohydrate media, Leach's type cultures A and L 1 and 2 were in all cases slower to form acid than were the cultures isolated by the writer. All of the characters as given by Leach were answered by 5 of the writer's isolates, namely, cultures SC 6, 13, and 14, and M 14 and 16.

It should be observed also that of the writer's blackleg cultures, C and M 6, 7, 15 and 16 were only weakly pathogenic on potato stems, although they caused a white soft rot.

It is of special interest to note the different sources of the writer's blackleg bacteria. Cultures SC 6 and 13 were from a decaying potato seed piece and spinach plant, respectively, that were infested with the seed-corn maggot. Culture SC 14 was from a potato plant affected with blackleg secured from South Carolina. Culture C was isolated from a decaying carrot root, and culture M 14 from potato scab lesions. Cultures M 15 and 16 were secured from the discolored vascular bundles of a potato tuber. It is obvious that the blackleg pathogen is often associated with diseased conditions other than potato blackleg.

According to the data included in tables 1 and 2 all of the blackleg and soft-rot pathogens were identical except in the production of gas on the 3 sugars and in the production of indole. The production of gas on the carbohydrates often has been considered an important determinative characteristic for the group (1 and 7); but many workers have shown gas production to be a variable and rather unreliable characteristic, especially when produced in small amounts. This is because of its ready absorption by the liquid medium. Harding and Morse (8, p. 269 and 279) found that the ability to produce gas on the carbohydrate media varied greatly from time to time for the same organisms. The writer's results also show that pathogens capable of producing blackleg vary as to their ability to produce gas. Some of the blackleg isolates have constantly produced gas on the carbohydrate media. Other blackleg cultures, however, both from South Carolina and from Maine, have been negative in this respect. Judging from these data it is felt that the ability to produce gas on the sugars is unsatisfactory as a means of establishing the blackleg and soft-rot groups of pathogens as different species.

Different writers in the past have been prone to create new bacterial species on rather minor cultural variations, differences in host relations, and symptoms. Different isolates of a bacterial pathogen often vary to some extent in minor characteristics. On the basis of such variations numerous new species have been created. There is now a great need to simplify the classification and the nomenclature of this group of bacteria. The increased amount of information available has shown that many of the previously described soft-rot and blackleg pathogens are identical or, at least, very similar. The variations that do exist between the described species of soft-rot bacteria often are not great enough to enable one to predict the symptoms incident upon inoculation. Furthermore, bacterial strains varying in certain cultural characteristics may produce the same disease symptoms. The writer, in view of these facts, agrees with Leach that the entire group should be included as one species consisting of numerous slightly variable strains. He states that the causal organism should be *Bacillus carotovorus* Jones, because of priority. However, in view of the earlier discussion of synonymy, *Erwinia carotovora* (Jones) S.A.B. is now the name preferred by the writer. Therefore, the soft-rot and blackleg pathogens studied by the writer comprise one bacterial group of which *Erwinia carotovora* is considered the type culture.

COMPARISON OF OTHER ISOLATES NOT BELONGING TO THE SOFT-ROT OR BLACKLEG GROUP

The other bacteria studied by the writer also may be classified into groups according to the different characteristics given in tables 1 and 2.

Those cultures possessing polar flagella and causing a yellow or cream-color decay of potato seed pieces in South Carolina comprise one natural group of isolates (SC 15 to 23). It is to be observed that all of these bacteria, excepting SC 23, which peptonizes litmus milk, have similar physiological as well as morphological characteristics. These organisms are all short rods with polar flagella, and do not produce capsules or endospores. They are Gram-negative and strictly aerobic, produce stratiform type of liquefaction in gelatin, and reduce nitrates slowly to nitrites without the formation of gas. They hydrolyze starch and cause alkaline reaction on the sugar media without gas production. They do not curdle litmus milk but produce a slight alkalinity. They do not produce indole or hydrogen sulphide.

Cultures SC 25 and M 31 are different from the group, SC 15 to 23, although all of these organisms produce similar symptoms on potato slices. The organisms SC 25 and M 31 are characterized by peritrichic flagella, which distinguish them from SC 15 to 23, which have polar flagella. Culture SC 25 appeared also to be a vascular parasite, and was isolated from the vascular bundles of potato stems far removed from the point of inoculation. Cultures SC 25 and M 31 have nearly identical physiological characteristics. They also are very similar physiologically to the group, SC 15 to 22 from South Carolina, except that SC 25 and M 31 have the ability to peptonize litmus milk.

A miscellaneous group includes those bacteria causing a slowly developing brownish decay on potato slices but are incapable of producing blackleg. These isolates differ from each other by certain physiological characteristics such as the relation to oxygen, the ability to ferment the different carbohydrates, and in the ability to peptonize litmus milk. This latter group, in addition to decaying seed pieces, also may influence the activities of the more virulent bacteria.

Other pathogenic organisms play a part in the destruction of potato seed planted in the South and in Maine. One of these was commonly encountered in soil and decaying seed pieces from South Carolina and Virginia. This organism was not studied in detail, but preliminary work indicates this organism is *Bacillus mesentericus* (Flügge) Migula. It is of interest in this connection that Brierley (5) found this organism the causal agent of a wound rot of potato tubers.

SOURCES OF ERWINIA CAROTOVORA

In the South, *Erwinia carotovora* was more frequently encountered as the causal organism of seed-piece decay than as a cause of blackleg. Under the conditions current in the South, the environment was so favorable for the development of rot that the seed pieces were destroyed before the sprouts could emerge and become large enough to be recognized as plants affected with blackleg. For this reason blackleg is reported less commonly in the southern potato regions than in the North.

The question naturally arises as to the source of the initial infection that results in the decay of the potato seed pieces and in blackleg. The soft-rot bacteria apparently are quite common and widely distributed, and were isolated from the potato and from several types of decaying vegetables both in the South and in Maine. Some of this infection undoubtedly comes from the soil, the pathogenic bacteria entering the host only under certain environmental conditions or when introduced by certain insect larvae. This appears to be particularly true in the South where the soil was found to contain numerous pathogenic bacteria and where insects are abundant. The writer feels that much of the seed-piece decay that occurs in this region is caused by contamination from the soil and not from the seed tubers.

Soft-rot bacteria were found present in the fungus mats of *Phoma tuberosa* occurring in seed potatoes and in the lesions caused by the common-scab and the powdery-scab organisms. They were isolated also from a tuber affected with late blight. Much of the decay that occurs in the bin is obviously the result of contamination following fungus decay of the seed tubers. These bacteria may be spread by the cutting knife, or by contact, to the freshly-cut surfaces of the seed pieces, but result in a decay of the seed piece or in blackleg only when subjected to certain environmental conditions. Other workers have shown that blackleg may be seed-borne. The writer, however, feels that this method of perpetuating the disease is of less importance than it is commonly thought to be.

The blackleg organism also was found to be the cause of the decay found associated with certain insects, including the seed-corn maggot, the cabbage maggot, the carrot rust fly, and a Staphylinid insect. In most cases, however, (with the potato at least) it would seem that the insects were attracted chiefly to those seed pieces that had previously been affected with superficial decay caused either by *Erwinia carotovora* or, more frequently, by certain less virulent bacteria.

The soft-rot and other pathogenic bacteria were isolated from the surface of eggs and from within the puparia of the seed-corn maggot and the cabbage. The soft-rot bacterium was isolated from the puparium of the cabbage maggot that had been exposed to a severe winter of Aroostook County, Maine, and there is no doubt that this insect, as well as the seed-corn maggot and the seed-potato maggot, may also serve to overwinter the soft-rot organism. This fact, however, may not be of much practical importance because the organism is present in the soil and elsewhere.

SUMMARY

Sixty-two bacterial organisms found associated with potato blackleg, seed-piece decay, and other soft-rot diseases, were studied. Isolates originating from different sources in Maine and South Carolina were compared and their morphological, physiological, and pathogenic characteristics were determined.

A number of bacteria, each causing both blackleg and soft rot of potato in Maine or South Carolina, were very similar, one to another, and were not distinguishable from authentic cultures of *Erwinia carotovora* (Jones) S.A.B. The cultures were identical in all of the physiological characteristics except in the production of gas on dextrose, sucrose, and lactose, and in the production of indole. Some cultures, both from Maine and from South Carolina, produced no gas. Others produced gas on all three sugars. Five cultures produced gas on lactose and sucrose but not on dextrose. It is thus seen that the ability to produce gas on the different carbohydrates is not a satisfactory criterion by which to separate and distinguish the soft-rot bacteria.

The isolates answering the morphological and physiological characteristics of *Erwinia carotovora* varied greatly in degree of pathogenicity. Some were only weakly parasitic, while others were quite virulent and destructive.

Bacteria capable of causing blackleg and seed-piece decay (a form of soft rot) were secured from a very wide range of sources. This lends support to the view that much blackleg and seed-piece decay may originate not from diseased seed stock, but from contamination that occurs after the seed tubers have been cut. The blackleg organism was found intimately associated with certain insects commonly present in decaying plant tissue. It was found capable of remaining viable within the puparia of the cabbage maggot after exposure to a severe northern Maine winter. It was very prevalent in the soil from Charleston, S. C.

The blackleg pathogen was sometimes isolated from plants with symptoms not typical for blackleg. These plants were very small with darkened and decayed stems. Such plants appear early in the season and are often not recognized as blackleg.

An undescribed organism with polar flagella was found to be responsible for a yellow seed-piece decay in the vicinity of Charleston, S. C.

In Maine still different bacteria may cause a similar yellow decay. Still other bacteria causing a rather slow, white to brownish decay may be responsible for the destruction of potato seed pieces in Maine fields. These bacteria were isolated from a variety of sources besides potato seed pieces, and appear to be the cause of certain soft rots that occur in members of the mustard family and probably in other plants.

Pathogenic bacteria were isolated from the discolored vascular bundles of tubers and from tubers affected with a "stem-end browning disease." These two bacteria are quite similar to each other.

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THE EFFICIENCY OF THE POURED PLATE TECHNIQUE AS APPLIED TO STUDYING BACTERIAL PLANT PATHOGENS[1]

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INTRODUCTION

Since the validity of research depends not only on the methods employed in the actual work but also on those applied in the interpretation of the results, it is not surprising that Ludwig long ago insisted "Die Methode ist Alles." Correspondingly, it is essential for the progress of science that the methods used be subjected repeatedly to critical examination.

Adequate experimental technique is particularly desirable if investigators of plant pathogens are to study important basic problems without becoming mired in the slough of contaminations and, therefore, discredited. Plant materials are admirably suited to the examination of fundamental problems in variability and pathogenicity, as has been pointed out elsewhere, (*e.g.*, Riker and Berge (12); Riker (10); and others). Some of the advantages plant materials have over animal are listed: (1) Large numbers of plants can be grown easily. (2) Their cost is relatively low. (3) A variety of disease types is available, including cell stimulation, rapid necrotic disintegration, vascular invasion, and "toxin" production. (4) Epidemics are easily induced without damage to public health. And most important of all, since pathogenicity must be defined in terms of relative resistance of the host, (5) plants are available that are genetically satisfactory. The progeny of long lines of selfed individuals insures uniformity in many readily available varieties. It is frequently possible, by means of vegetative propagation, to secure any number of experimental, genetically identical plants.

With these splendid opportunities for basic work, it is obviously essential that the purity of the pathogenic cultures used be placed, as far as possible, beyond question. Most workers agree that fungus and bacterial cultures should be derived from single cells if their purity is to be reasonably above suspicion. Single spores or hyphal tips of fungi are large enough to be isolated with relative ease. However, with bacteria there is greater difficulty. Correspondingly, there is often a tendency to place dependence for pure cultures[3] on the simple poured-plate technique. The purpose of the present paper is to consider how trustworthy this procedure is with common plant pathogens.

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[1] Approved for publication by the director of the Wisconsin Agricultural Experiment Station.

The senior writer presented in discussion at the Indianapolis science meetings much of the material in the present paper. This report, together with some new data, was prepared for publication by request.

[2] The writers are indebted to R. B. Kershner, Department of Mathematics, University of Wisconsin, for the mathematics in this paper.

[3] The term "pure culture" is used to designate a culture free from admixture with other strains of the same species, as well as free from other species.

DILUTION PLATES

The poured-plate technique has several well-known major variations. It is usual to make successive dilutions of a bacterial suspension in melted nutrient agar at about 45° C. The agar is moved so as to distribute the bacteria and is allowed to solidify in Petri dishes. The manner of making dilutions, *et cetera,* whether by pipette in test tube, transfer loop in Petri dishes, or other means, is varied according to the requirements and convenience of the technician. A common procedure, with plant bacteria, is to macerate a small piece of diseased tissue containing bacteria in several cubic centimeters of liquid, such as distilled water or nutrient broth, and to make successive loop dilutions from this material into 3 agar plates, respectively. Under these circumstances it is obvious that the bacteria, having occurred for the most part in a densely compact mass in the plant tissue, may diffuse as individuals into the liquid but also may remain in more or less definite clumps in the resulting suspension. The size and nature of the clumps depend on a variety of factors. After a suitable incubation period colonies develop that often may represent uncontaminated cultures of the organism sought. Cultures from such colonies are satisfactory for many types of work. The chances for purity in such cultures are better if the diseased tissue comes from the most advanced margin of a rapidly spreading lesion, since, in the plant, a strongly virulent pathogen may grow away from secondary organisms or even from less virulent mixtures of the same species.

Although the poured-plate technique has contributed to progress for over 50 years, bacteriologists long ago began searching for improvements in method, as they worked on more critical problems. For example, when only a few of the desired bacteria are present in a general mixture, it can often be placed in a liquid medium more favorable to the desired few than to the others. A series of successive transfers will provide *enrichment* of the desired organisms, making subsequent isolations from poured plates much easier. One of the best modifications[4] of the dilution-plate procedure is as follows: The culture to be purified is grown under favorable conditions until it is in the "logarithmic growth phase," which, for many plant pathogens, is 12 to 24 hours at 24° C. Pellicle and sediment commonly appear soon, *e.g.*, after 36 to 48 hours. With control over various critical factors that might induce clumping, successive dilutions are made, respectively, in several lots of favorable liquid medium and then mixed with melted and cooled agar in Petri dishes. When colonies develop they are selected for transfer only if a relatively small number appear in a plate and after they have been examined under suitable magnification to avoid any visible evidence of mixture in the colonies. This process is repeated several times. This modification is much superior to the ordinary poured-plate technique and the two should not be confused.

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[4] While somewhat beyond the scope of this paper, attention is directed to the use of "surface plating," valuable not only for counting bacteria but also for securing excellent distribution of colonies. Reyniers, A. J., Mechanising the viable count. Jour. Path. Bact. 40: 437–454. 1935.

The more essential features influencing the purity of cultures secured from single colonies with the poured-plate technique center about (1) the dispersion and growth of bacteria in the agar medium, (2) the minimum distance two or more growing cells must be separated if they are to form single colonies, and (3) the merging of colonies initially distinct but close enough together so that they coalesce as they grow. These items are considered very briefly in order.

DISPERSION AND GROWTH

Satisfactory dispersion in various types of media and subsequent growth of most of the bacteria can be secured, as shown by various workers. For example, McNew (5, 6) has demonstrated that, when adequate precautions are taken, such dispersion and growth can take place in agar. However, if such precautions are neglected, the situation is quite different. When bacteria have become embedded in mutual gum, as the plant pathogens usually do, both in host tissue and in older cultures, the bacterial clumps are sometimes difficult to disperse satisfactorily. The numerous factors[5] influencing bacterial dispersion in liquids have received study too voluminous for consideration here. The work is reviewed by Buchanan and Fulmer (1). It is apparent that the poured-plate technique, without important precautions, is apt to allow a number of bacterial clumps in the dilution plates. When clumps are present they commonly grow rapidly and suppress the development of isolated cells.

SINGLE COLONIES

The question of what constitutes a bacterial "clump" or single "locus" needs clarification. The expression is used in this paper in the sense that only cells, practically touching, side by side, or end to end, are in a single locus, and that, otherwise, they are not in the same locus. Thus a locus may contain either a single cell or a clump of cells. This concept seems satisfactory from the standpoint of dispersion, but needs to be clearly distinguished from the situation when growth of the bacteria is involved. For example, 2 surface cells growing at the same rate may be separated by 5, 10, 50, or 100 microns, or even by many more, and still merge into a single colony with no visible evidence of mixture. When there is a difference in rate of growth, opportunity for invisible merging is increased. Thus, if one bacterium grows rapidly and a second grows slowly (because of its nature or of its location just below the surface) the rapidly growing colony may overspread the slower one and combine with it, leaving no visible evidence. The possibility of mixtures that are visible may be observed by examining under suitable magnification a situation that is conspicuous.

COALESCING COLONIES

The opportunities for colony mixtures are illustrated in figure 1, A. A

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[5] For example, apparently any factor that lowers the electric charge of bacteria may cause them to clump together. Such factors may be found among acids, salts, colloids, sensitizing substances, and agglutinins.

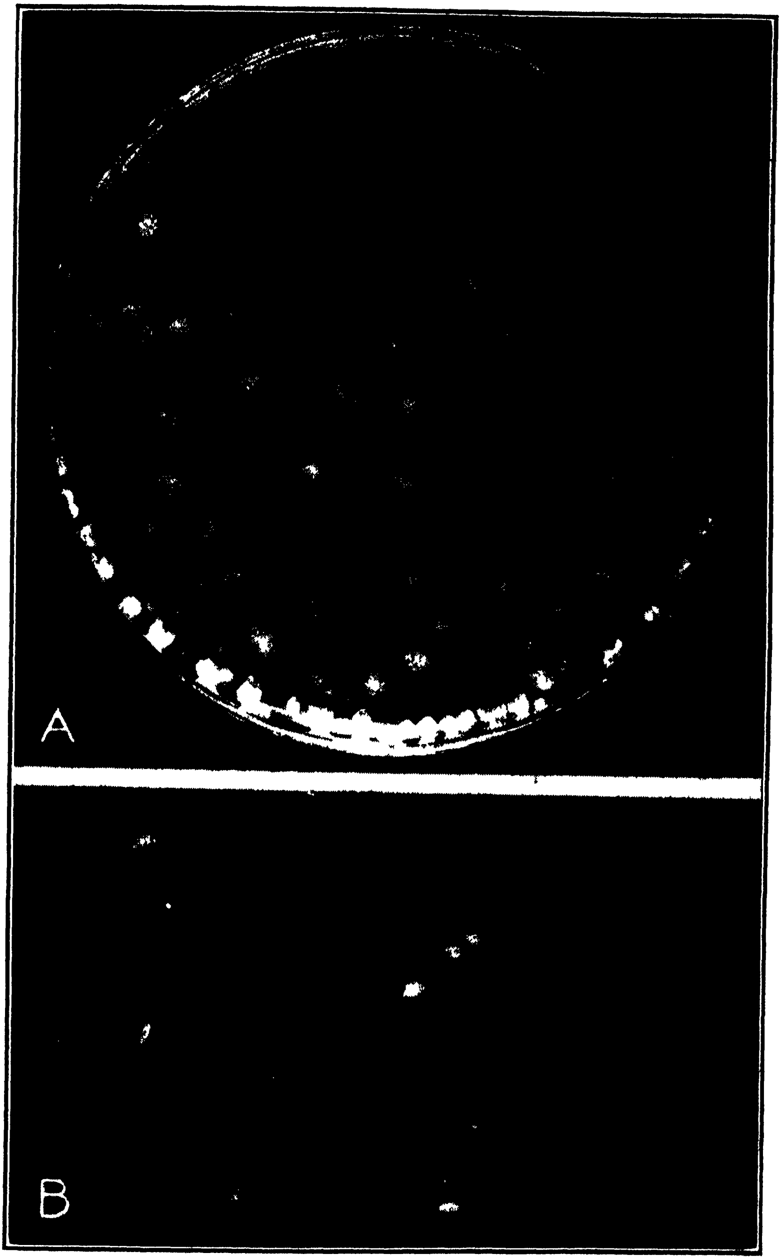

FIG. 1. Dilution plate showing visible mixtures of colonies. A. A number of round but a much larger number of irregular colonies appear. The irregular colonies, for example, (a) are formed by two or more colonies growing together. B. One of the round colonies photographed with low magnification. The view is through the bottom of the dish and with a beam of light from a sharp angle, so that deep colonies cast shadows indicating their depth. Colonies marked *b* are against the glass; those marked *c* are in the agar; and those marked *d* are apparently growing into the surface colony. Photographs by Eugene Herrling.

dilution plate was poured with beef-extract-peptone-glucose agar containing organisms from a bacterial blight lesion on a bean pod. Ordinary technique was employed. The Petri dish was incubated for 2 days at 75° F. In all, 112 surface colonies resulted. In this example, when 2 or more colonies have run together, as in figure 1, A, a, they are counted as one. There are 86 of this kind, and only 26 that are not obvious mixtures. If these 26 are examined with magnification it appears that a number of them cover sub-surface colonies that are potential sources of mixture. One such surface colony was photographed (Fig. 1, B) through the agar at the bottom of the plate. A beam of light from a sharp angle was employed. There are, under this surface colony, 3 very deep colonies (b) that have spread out against the glass. There are 3 sub-surface colonies (c) that, from the shadow they cast, appear to be deep enough in the agar not to be mixed with the surface colony. There are, however, 2 others (d) almost at the surface, which, apparently, cast little or no shadow, and seem to be touching the surface colony. Only 3 of the 26 round surface colonies do not cover deep colonies. While surface and deep colonies do not necessarily mix, it is safer to recognize that they may. Thus, there is direct visual evidence to cast doubt upon the purity of all but 3 of the 112 surface colonies. Obviously, an experienced bacteriologist would choose a higher dilution or at least make transfers only from these 3 colonies. However, there is no assurance of the purity of the cultures isolated from any of these three. The mixtures illustrated here are visible, but doubtless mixtures occur that are not visible even under magnification. While some of the difficulties appearing in this case in relation to purity of cultures may be avoided by making a somewhat higher dilution, this type of plate is all too common in isolations with the ordinary poured-plate technique.

PROBABILITY

The mathematical chances for colony mixtures involved in the above example are briefly examined. If one considers merely the possibility of surface colonies coalescing, only 26 out of 112 colonies were not visibly mixtures. In a random selection of these colonies the chances are 23 in 100 of avoiding an obvious mixture. Five successive platings would improve the chances approximately to 72 in 100. However, only 3 of the surface colonies that were not obviously mixed with others failed to cover deep colonies. When the latter are considered, there are approximately 2.8 chances in 100 that a random selection of a colony would hit one not visually under suspicion of being a mixture of colonies. Five successive trials on this basis would improve the chances to approximately 11 out of 100, but the odds still favor the picking of a questionable colony. The size of the colonies and the number per plate strongly influence any probability involved, as brought out later.

Some indications of the probabilities that colonies may coalesce in poured plates may be obtained from a consideration that seems not to have been given in the bacteriological literature. The writers are indebted to R. B. Kershner for the mathematics involved. While this kind of study might be greatly extended, it is given here in only its most elementary aspects. If one selects a given surface locus from which a colony has grown, it is possible to estimate the chances that this colony, during its growth, may have coalesced with another colony starting from a different locus. As a rough approximation let the colonies be considered as small circles with uniform diameters and distributed at random within a large circle, the Petri dish. The probability (chances in one, by definition) that one small circle among a number distributed at random within a large circle, would not meet another small circle is

$$\left(1-\frac{(2d)^2}{D^2}\right)^{n-1}$$

where n is the number of small circles, d the diameter of the small circles and D the diameter of the large circle. An indication of the variation of this probability may be secured from table 1.

TABLE 1.—*Probability that a given small circle in a large circle will not touch another small circle*

Diameter (d) of small circle[a]	Probability with given number (n) of small circles per large circle with 90 mm. diameter (D)					
	10	20	50	100	200	400
mm.						
1.5	.990	.98	.95	.89	.80	.64
3	.96	.91	.80	.64	.41	.17
6	.84	.70	.40	.17	.03	.0007

[a] The probability given by the formula is transferred to chances in 100 by moving the decimal point two places to the right. Thus, when there are 50 small circles 6 mm. in diameter distributed in a large 90 mm. circle, a small circle selected at random will fail to touch another small circle 40 times out of 100.

This approximation is very rough. For instance, it assumes that all colonies are of the same size. This assumption is rather inadequate under several conditions, for example, in case there are several different kinds of bacteria present that may have different rates of growth. The results are too conservative, since they take no account of the fact that many coalescing colonies obviously are combined and no transfer would be made from these mixtures by an experienced bacteriologist.

A somewhat better approximation which errs in being, if anything, too encouraging, but seems to come closer to representing the actual situation, can be arrived at as follows. Let the colony under consideration grown from one locus be considered as a circle of diameter d. Then if another of the original growing loci with which the plate was seeded lies in that circle the colony is certainly not derived from a single locus. Now the probability (or chances in one) that one point among a number distributed at random in a large circle should fall within a given small circle is

$$\left(1-\frac{d^2}{D^2}\right)^{n-1}$$

where n is the number of points, d the diameter of the small circle, and D the diameter of the large circle. An indication of the variability of this probability may be secured from table 2.

TABLE 2.—*Probability that one point among a number distributed at random in a large circle will fall within a given small circle*

Diameter (d) of small circle	Probability with given number (n) of original loci per large circle with 90 mm. diameter (D)					
	10	20	50	100	200	400
mm.						
3	.990	.98	.95	.89	.80	.64
6	.96	.91	.80	.64	.41	.17
12	.84	.70	.40	.17	.03	.0007

It is important to notice that in applying these tables to the problem at hand the number n must be the number of original loci with which the plate was seeded and, correspondingly, is somewhat higher than the colony count. It must also be kept in mind that table 2 can be considered as giving the probability that a given colony is unmixed only in case this colony is not visibly formed by coalescing colonies, in which case the assumption that the colony is a circle is very nearly satisfied. It is clear that various factors such as variability in colony size, different rates of growth, and departure from random distribution would modify any direct inference.

An indication of the application of this probability may be secured from a few examples. If 50 surface colony-producing loci were placed in a 90-mm. Petri dish and the colonies grew to 6-mm. diameter, the probability from table 2 is approximately .80 (or 80 chances in 100) that a given round colony came from a single locus. Thus 20 per cent of the loci probably led to mixed colonies. Also, if 100 surface colony-producing loci were placed in a 90-mm. Petri dish and the colonies grew to 3-mm. diameter, the probability from table 2 is approximately .89 (or 89 chances in 100) that a given round colony came from a single locus. Thus 11 per cent of the loci led to mixed colonies.

So far the estimates have been based on the number of original colony producing loci. If one approaches the problem now from the number and size of colonies in a Petri dish, it is possible to estimate (1) the percentage of surface colonies grown from more than one locus and (2) the number of original surface loci, but the mathematics involved is beyond the scope of the present paper. A suggestion about the results of such an estimate can be seen from the following. If one starts with 100 surface loci and allows growth until the colonies are approximately 3 mm. in diameter then, according to table 2, approximately 89 pure colonies will result. The remaining 11 loci will merge in growth and form about 5 colonies of which 4 came from the merging of 2 colonies and 1 from the merging of 3 colonies. Thus a plate containing 94 colonies of 3-mm. diameter approaches 89 pure colonies and 5 mixed colonies.

On the basis of the chances shown in these tables, it is clear that the smaller the colonies and the fewer per plate the less chance there is for mixing. The probability falls off very rapidly as either the size or number of colonies increases. In relation to practical considerations, it may be recalled that, when colonies are crowded, the colony size is reduced. Conversely, when a small number of colonies appears on a favorable medium, they grow rapidly and cover a relatively large area. For example, under favorable conditions four gall-inducing species of bacteria produced colonies with diameters between 20 and 28 mm. in diameter in 14 days (Pinckard (8)). Many plant pathogens when well separated and incubated several days under favorable conditions produce colonies 5 to 8 mm. in diameter.

Other applications of this mathematical approach are relatively obvious. In counting bacteria there has been difficulty in explaining the discrepancy between direct microscopic counts and colony counts made on dilution plates. Some of this discrepancy as related to surface colonies can be clarified by reference to table 2. A correction of data from plate counts on this basis might bring them somewhat closer to those from microscopic counts. The same kind of corrections might apply to estimates made by the local lesion method on the number of virus particles in a liquid. Inferences similar to those about the "purity" of a bacterial culture derived from a colony can be made about the "purity" of a virus separated from others in a mixture by this local lesion technique.

Important as questions of probability unquestionably are, it is likewise desirable to consider the general problem in relation to experimental manipulations.

ACTUAL EXPERIENCE

Comparative trials with poured plates and single-cell technique have been reported for several plant bacteria. Nirula (7) found certain cultures still to be mixed after a number of successive dilution-plate isolations. Baldwin (unpublished) found that pea and clover bacteria were separated with certainty only by single-cell isolations. Riker *et al.* (11) and Wright *et al.* (15) purified 31 cultures by 4 successive dilution plates with each. They reported that plates were seeded from vigorous 18- to 20-hour-old cultures and the dilutions were made in the liquid medium used. Only plates which showed less than 50 colonies were used for making sub-cultures. Before transfers were made a selected colony was examined under the microscope for evidence of mixture with another. With all this care, 29 of the 31 cultures seemed pure and appeared to fall either into a crown-gall group or a hairy-root group which were distinguished by more than a dozen means. But 2 cultures still showed intermediate characters and, as explained below, were examined with single-cell technique.

Interpretations based on cultures from poured-plate colonies may require critical examination. Three examples may be mentioned.

First, Riker *et al.* considered two of their single-colony cultures to be intermediate and all cultures involved to belong to a single species having great variability. However, this attitude was later changed (Riker *et al.*

(11); and Wright *et al.* (15)) when, by means of single-cell technique, an intermediate culture was separated into its components. Thus the variability was not inherent in the cultures, but was owing to a mixture.

The second example is concerned with the increase and decrease of virulence in *Phytomonas stewartii* (E.F.S.) Bergey *et al.* as a result of growth in resistant and susceptible maize plants. Two workers have recently approached the problem with different technique. Wellhausen (13) began with single-colony cultures and his procedure was as follows, "Two plants per culture from the 15 inoculated were taken for reisolation. Upon reisolation, 10–15 colonies per culture were picked from the plates at random and transferred to agar slants. The progeny of these 10–15 colonies pooled constituted the inoculum for the next passage." He found that successive passages through resistant hosts increased virulence for corn, while successive passages through susceptible plants decreased virulence. He concluded, "At this stage of the investigation it is impossible to give a final interpretation of the methods whereby the above changes were brought about. It may be that the host environment has a direct effect on the organism, bringing about temporary changes that may last through several generations. On the other hand, the changes in the various strains may have come about through random mutation and natural selections of those organisms best adapted to the particular environment of the host. . . . The fact that virulence may be increased in a resistant host and decreased in a susceptible host of a particular species may have an important bearing on the rise and decline of disease epidemics in that species." An additional explanation might perhaps be merely a differential selection made by the host plant from a mixed population. Such a selection was found by Lincoln (4), who has worked on this problem with single-cell cultures and reported, "Upon passage through the susceptible host there is a differential selection for the avirulent type of bacteria. For example, beginning with a 50–50 proportion of virulent to avirulent bacteria, the proportion observed after a 15-day passage averaged 39–61. . . . Passage through the resistant host results in a differential selection for the virulent type of bacteria, instead of the avirulent, as in the experiment above. When the initial proportion of virulent to avirulent bacteria was 50–50, the proportion observed after a 15-day passage averaged 63–37."

The third example is taken from Winogradsky (14), who discusses mistakes resulting from contaminations in studies of pleomorphism in Azotobacter. He says, "More interesting are the errors mentioned, which should be discussed here in some detail. The source of them lies in the fact that the cultures of Azotobacter, isolated *lege artis* and qualified to be pure, become spontaneously impure with time. The cause of this annoying property is not difficult to understand. It is due to a fundamental imperfection of the standard method of isolation by the plate technique. The method is reliable only in the case where the species to be dissociated grow almost equally well on the medium employed. As soon as there are any appreciable differences in the rates of colony formation, the isolation becomes less and less dependable, because of the presence of latent germs. The latter, when altogether unable to grow on the medium, render the success of the operation wholly uncertain. The case of the nitrifying bacteria, where isolation blunders occurred so often in the course of nearly fifty years, can be cited as one of the best examples of this uncertainty. Among many others, the example of Azotobacter is also one of the most instructive, for it is not difficult to detect all of the factors of its unreliability." He continues, "This behavior of Azotobacter cultures, becoming impure with age in spite of most careful purification, the writer has frequently had occasion to observe with his own cultures as well as with those obtained from abroad through the courtesy of fellow bacteriologists. Nearly 30 per cent of them, or even more, became impure, although there could be no possible doubt concerning the perfect execution of the standard operations."

It is recognized that the results with single-colony cultures and single-cell cultures are frequently identical, as often described, indicating that the single-colony cultures in such cases are "pure" cultures. However, there are differences frequently enough to require caution for exacting study. The poured-plate technique, even in skillful hands, thus appears inadequate for certain critical work, such as investigations on the variability of bacteria.

SINGLE-CELL ISOLATIONS

The need for a means of isolating and growing single bacterial cells under observation has resulted in a considerable literature too large for examination here. It is reviewed, for example, by Dickinson (2), Reyniers (9), and Hildebrand (3). Methods are well enough developed that various single-cell techniques are routine in many bacteriological laboratories.

The use of single-cell cultures is, obviously, only one phase of pure-culture technique. Such cultures can be contaminated just as any other cultures. Likewise, just as there are numerous opportunities for error with the poured-plate methods, the value of the single-cell methods depends on the skill of the operator. Learning to make single-cell cultures is somewhat like learning to drive an automobile—some people have no difficulty, while others never become skillful. The beginner with single-cell procedure commonly feels lack of confidence in being able to see the individual cells and to determine that only one is there. So many factors influence the outcome that such beginners may well seek instruction from an experienced technician rather than depend on printed directions. When a single cell has been isolated in a hanging drop, on a film of agar, or on a sheet of cellophane, it is wise frequently to observe the preparation during growth and thus to check whether any otherwise unseen form also is developing.

Single-cell technique is no panacea for pure-culture work, even though it is a distinct advance over the best poured-plate methods. As already explained (*e.g.* Riker *et al.* (11)) there is always danger that more than one cell may be carried over in the procedure, especially if it is very small. The possibility of gonidial forms and even of an ultramicroscopic stage are also to be held in mind.

Even with the various drawbacks to single-cell isolations, the large amount of evidence accumulated points to the necessity of supplementing single-colony isolations with single-cell isolations for exacting investigations. If one is to spend months and years studying a particular culture, the time spent in making sure of its purity, as far as possible, adds greatly to the validity of the work. At the present time few research men would have the temerity to report, for example, on bacterial variations, without the relative security of single-cell isolations. For such studies the difficulties are so great that even the best methods available are none too good.

The writers hope the data and discussion in this paper will stimulate younger investigators not only to study basic problems involved in pathogenicity and variability, thus making use of the splendid opportunity offered by plant materials, but also to be satisfied with only the best available technique in their investigations.

SUMMARY

The ordinary poured-plate technique, while generally useful, appears inadequate for critical bacteriological studies, particularly where strain variations are involved.

The common poured-plate technique is considered insufficient because: (1) Unless special precautions are taken, many of the colonies which develop come from bacterial clumps. (2) A large percentage of the colonies may run together in commonly employed dilutions. While many such mixtures are readily seen, doubtless a number of others are not visible. (3) Practical experience has shown that important plant bacteria are not always separated by dilution plates, even with elaborate precautions.

Modifications of the poured-plate technique, which take care against clumping of bacteria before or while dilution plates are poured in suitable media, and which employ satisfactorily high dilutions and rigid selection under magnification, are much better than the ordinary procedure.

A brief mathematical study estimates the probability that the growth from original bacterial loci may coalesce as the colonies develop. The more colonies per plate and the larger their size the greater chance there is for their coalescing.

This probability may have applications, for example, to bacterial plate counts and to the local-lesion method for studying plant viruses.

The single-cell isolations give greater assurance of pure cultures than do even well-controlled dilution plates. Both methods, however, have their advantages, and may well be used to supplement each other.

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REINOCULATION OF RESISTANT VARIETIES OF WHEAT WITH PURIFIED PHYSIOLOGIC RACES OF TILLETIA TRITICI AND T. LEVIS[1]

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INTRODUCTION

It has been reported by a number of workers (1, 2, 4) that the virulence of the bunt fungi, *Tilletia tritici* (Bjerk.) Wint. and *T. levis* Kühn may be increased by taking inoculum from a resistant variety of wheat and reinoculating that variety with its own bunt. Bressman (1) inoculated the variety Ridit with its own bunt and succeeded in increasing the percentage of infection from 2.5 the first year to 73.4 the following year. He suggested that this increase in susceptibility might have been due either to mutation or the varietal selection of a Ridit race from a hybrid or from a mixture of races in

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[2] Assistant Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. The writer acknowledges his indebtedness to H. A. Rodenhiser, of the Division of Cereal Crops and Diseases, at whose suggestion the research was undertaken, and to C. S. Holton, of the same Division, for supplying the inoculum of the purified physiologic races of bunt employed in the experiment.

which the Ridit-susceptible race appeared in such small proportion the first year that only a low percentage of infection occurred. In other words, his conclusions suggested that there was a straining out of the inoculum not capable of infecting Ridit and a concentration of the Ridit-susceptible inoculum. Dillon Weston (5) reinoculated the variety Sherman with its own bunt and obtained an increase in the percentage of infection from 1.0 to 85.7. He obtained similar results when the varieties Ridit, Turkey, Hussar, and Berkeley Rock were inoculated, respectively, with chlamydospores of *Tilletia tritici* that had been collected from these varieties. Melchers' (3) work with *T. levis* produced similar results when resistant varieties were inoculated with their own bunt. He increased the percentage of bunt on Banner Berkeley from 23.1 to 57.9, on Hussar from 7.4 to 47.2, and on White Odessa from 1.1 to 37.9. The following year, however, these same varieties inoculated with their own bunt produced only 27.9, 16.6, and 15.5 per cent infection, a reduction of 30, 30.6, and 22.4 per cent, respectively. Melchers attributed this reduction in percentage of bunt to environmental conditions at the time of seeding. He failed, however, to make any mention of the cause of the low infection the first year, which also might have been due to environmental conditions. His results, therefore, suggest that the increase in percentage of bunt may have been due to the effects of the straining out of the inoculum to which the varieties were resistant in combination with adverse environmental conditions rather than to the "breaking down" of the resistance of the varieties. Flor (2) studied the screening effect of resistant varieties and found that pathogenically distinct races were readily separated in this manner. Varietal screening, however, had no effect on the pathogenicity of a collection propagated on a resistant variety, such as Hussar, for several years.

A higher infection was obtained in resistant varieties by reinoculation with their own bunt in each of the investigations cited above. It is significant, however, that in only one case, Flor's inoculum from Hussar, was a purified race[3] of inoculum used. Infection from this purified race did not increase on reinoculation to Hussar. In all probability each of the original collections contained more than one race. Thus, reinoculations over a period of 2 or 3 years probably resulted in the elimination of biotypes that were somewhat nonvirulent on the resistant varieties inoculated and in a concentration of those capable of infecting the variety. Since environmental factors during the infection period also play an important part in the amount of infection obtained from year to year, it is assumed that near-optimum conditions for infection prevailed during these experiments. Consequently, the combination of a concentration of biotypes capable of infecting a variety, together with optimum environmental conditions during the period of infection, would naturally increase the percentage of bunt,

[3] "Purified race" as used in this paper refers to a particular race that is always collected from a given variety. For example, in these experiments T-9 was always taken from Hohenheimer, T-8 from Albit, L-8 from Oro, L-6 from Albit, etc. Thus they may be considered pure for those biotypes that attack either Hohenheimer, Albit, or Oro.

and this increase might occur under conditions that would indicate its dependence upon a "break down" in the resistance of a variety.

Investigations were begun in 1935 at the Idaho Experiment Station to determine whether the resistance of a given variety could be reduced by repeated reinoculations with spores of a purified race taken from that variety. It was thought an experiment of this type would definitely prove if there was a straining effect when resistant varieties of wheat were repeatedly reinoculated with their own bunt or whether the increase in percentage of bunt was attributable to other factors.

MATERIALS AND METHODS

The bunt inoculum was secured from C. S. Holton, Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. The inoculum comprised 6 purified physiologic races of *Tilletia tritici,* 3 of *T. levis,* and a species hybrid. The races were taken at the commencement of the experiment in 1934 from each of the respective varieties on which they were to be used later. Following are the races as described by Rodenhiser and Holton (4): *T. tritici,* T–6, T–8, T–9, T–10, T–11, and 2 numberless races; *T. levis,* L–6, L–8, and an unnumbered race; and a hybrid between a race of *T. tritici* that infects Hybrid 128 but not Turkey, and a race of *T. levis* that infects both of these varieties. This hybrid was used for the purpose of indicating what might take place if hybridization occurred at any time during the course of the experiment. The following varieties of wheat were used: Hybrid 128 (C. I.[4] 4512), Ridit (C. I. 6703), Oro (C. I. 8220), Hussar (C. I. 4843), Turkey (C. I. 6175), White Odessa (C. I. 4655), Martin (C. I. 4463), and Albit (C. I. 8275).

Seed of these varieties was treated by the standard formaldehyde dip method, washed thoroughly with water, and allowed to dry. Seed for the subsequent years' use was harvested from the experimental rows so that the seed used for the entire test was progeny of the original seed. Powdered inoculum from each race was dusted on the seed at the rate of 0.3 g. of inoculum to 100 g. of seed. The seed was sown at the rate of 3 g. per 5-ft. row and the rows were 14 inches apart. In order to determine the percentage of soil infestation, nonsmutted susceptible Hybrid 128 was sown at 5-row intervals. Three replications were sown each year. The percentage of infection is based on the average percentage of heads infected in the 3 replications. The total number of heads per row averaged 265, 269, and 232, respectively, per year.

Inoculuum of each race was taken from (a) a susceptible variety and put on a susceptible variety, (b) a susceptible variety and put on one or more resistant varieties, (c) each resistant variety and put back on it, and (d) each resistant variety and put on a susceptible variety. Cross inoculations of this type made possible the checking of the viability of the inoculum as well as comparing the effect of reinoculating a resistant variety with its

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[4] C. I. refers to accession number of the Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

TABLE 1.—*Percentage of bunt obtained over 3-year period when susceptible and resistant varieties of wheat were reinoculated with purified physiologic races of Tilletia tritici and T. levis, Moscow, Idaho, 1935–37*

Wheat variety from which inoculum was taken	Wheat variety inoculated	Percentage of bunt											
		1935 Replication			1936 Replication			1937 Replication			Average		
		1	2	3	1	2	3	1	2	3	1935	1936	1937
		T. tritici, Race T-6											
Hybrid 128	Hybrid 128	51	36	47	71	81	86	51	58	42	45	76	50
Hybrid 128	Oro	0	0	0	0.3	3	1	0.5	0.4	0.4	0	1	0.4
Oro	Oro	0.3	1	0.3	2	0	2	1	1	0	0.5	1	0.7
Oro	Hybrid 128	70	66	39	80	81	91	53	77	64	58	84	65
Hybrid 128	Hussar	0	1	0	0	0	0	0	1	0.5	0	0	1
Hussar	Hussar	0	0	0.3	1	0	0	0	0.5	2	0.1	0.3	1
Hussar	Hybrid 128	50	67	54	81	75	85	56	50	82	57	80	63
		T. tritici, Race T-8											
Hybrid 128	Hybrid 128	69	87	53	75	93	94	70	72	52	70	87	65
Hybrid 128	Ridit	1	7	2	2	2	2	11	6	2	3	2	6
Ridit	Ridit	1	0.5	6	0	2	4	7	1	3	3	2	4
Ridit	Hybrid 128	63	79	77	88	87	95	75	72	86	73	90	78
Hybrid 128	Oro	4	4	3	10	3	2	3	2	2	4	5	2
Oro	Oro	5	4	9	1	2	4	2	4	1	6	2	2
Oro	Hybrid 128	59	77	55	81	94	58	71	71	73	64	78	71
		T. tritici, Race T-9											
Hybrid 128	Hybrid 128	29	24	31	90	68	95	51	92	57	28	84	67
Hybrid 128	Turkey	15	3	1	2	0.4	2	2	4	2	6	1	3
Turkey	Turkey	15	11	4	2	7	1	4	0.3	3	10	3	3
Turkey	Hybrid 128	49	41	45	75	72	80	60	73	59	45	76	64
Hybrid 128	Oro	0.3	0.4	1	2	1	0.4	0	1	2	1	1	1
Oro	Oro	0.3	3	0.3	[a]	[a]	[a]	0	1	2	1	[a]	1
Oro	Hybrid 128	52	40	35	91	88	71	63	66	71	42	83	67
Hybrid 128	Ridit	2	0	0	3	1	1	3	0	1	1	2	1
Ridit	Ridit	2	0	0	3	2	0	0	1	0	1	2	0.3
Ridit	Hybrid 128	29	51	35	86	96	77	4	61	78	38	86	48

[a] Data omitted because bunted plants were found, on microscopic examination, to contain *Tilletia levis* as well as *T. tritici.*

TABLE 1.—(*Continued*)

Wheat variety from which inoculum was taken	Wheat variety inoculated	Percentage of bunt											
		1935 Replication			1936 Replication			1937 Replication			Average		
		1	2	3	1	2	3	1	2	3	1935	1936	1937
T. tritici, Race T-10													
Hybrid 128	Hybrid 128	55	60	73	85	86	58	79	64	79	61	70	74
Hybrid 128	White Odessa	0.0	0.0	0.0	1	0.0	0.4	1	0.0	0.4	0.0	0.4	0.4
White Odessa	White Odessa	1	3	0.4	[a]	[a]	[a]	0	0.0	1	1	[a]	[a]
White Odessa	Hybrid 128	50	50	41	81	81	83	12	59	64	47	82	45
Hybrid 128	Ridit	2	0.0	0.0	3	5	5	0.0	0.0	0.0	1	4	0.0
Ridit	Ridit	0.0	2	0.0	1	0.0	0.0	1	0.4	0.0	1	0.3	0.4
Ridit	Hybrid 128	67	60	80	91	91	94	61	77	63	69	92	67
Hybrid 128	Turkey	4	11	8	1	3	2	5	6	9	7	2	7
Turkey	Turkey	13	15	3	3	2	2	1	6	0.4	10	3	2
Turkey	Hybrid 128	51	55	64	90	89	81	50	77	58	57	87	61
Hybrid 128	Oro	1	1	4	2	2	1	0.0	1	0.0	2	2	0.3
Oro	Oro	0.0	0.0	4	0.3	4	0	0.0	0.0	1	1	1	0.3
Oro	Hybrid 128	52	81	56	89	73	88	55	78	59	66	83	64
T. tritici (Nonnumbered race)													
Hybrid 128	Hybrid 128	45	67	50	84	77	94	52	65	70	54	85	62
Hybrid 128	Martin	36	43	52	95	80	93	61	56	52	43	89	56
Martin	Martin	22	52	48	92	92	94	36	59	60	40	93	51
Martin	Hybrid 128	51	66	60	87	95	92	54	77	61	59	91	64
Hybrid 128	Albit	40	70	44	93	98	99	39	63	68	51	97	57
Albit	Albit	58	63	22	86	100	93	54	56	71	48	93	60
Albit	Hybrid 128	92	52	91	90	80	92	80	76	77	78	87	78
Hybrid 128	Oro	3	7	4	20	17	17	8	3	4	5	18	5
Oro	Oro	12	1	2	16	12	9	10	4	6	5	12	7
Oro	Hybrid 128	53	76	4	80	91	69	73	72	69	44	80	71
Hybrid 128	Ridit	2	5	0.0	2	3	3	5	3	6	2	4	5
Ridit	Ridit	11	9	3	4	12	7	3	7	6	8	8	5
Ridit	Hybrid 128	72	59	55	88	93	95	76	62	58	62	92	65

[a] Data omitted because bunted plants were found, on microscopic examination, to contain *Tilletia levis* as well as *T. tritici*.

TABLE 1.—(*Continued*)

Wheat variety from which inoculum was taken	Wheat variety inoculated	Percentage of bunt											
		1935 Replication			1936 Replication			1937 Replication			Average		
		1	2	3	1	2	3	1	2	3	1935	1936	1937
		T. tritici (Nonnumbered race)											
Hybrid 128	Hybrid 128	53	71	55	93	93	82	66	75	75	59	89	72
Hybrid 128	Oro	14	3	4	11	19	12	6	6	2	7	14	5
Oro	Oro	13	8	1	16	22	11	5	8	4	7	16	6
Oro	Hybrid 128	76	68	65	96	94	88	65	70	66	70	93	67
Hybrid 128	Ridit	3	1	1	6	5	6	3	5	13	2	6	7
Ridit	Ridit	5	1	2	10	7	2	5	4	7	3	6	5
Ridit	Hybrid 128	75	41	57	92	94	96	77	79	64	58	94	73
		T. levis, Race L–6											
Hybrid 128	Hybrid 128	52	63	30	86	95	88	76	72	30	48	90	59
Hybrid 128	Ridit	0	4	2	3	4	0.3	5	5	8	2	2	6
Ridit	Ridit	2	1	1	3	3	4	3	1	6	1	3	3
Ridit	Hybrid 128	49	42	41	82	89	92	66	58	68	44	88	64
Hybrid 128	Oro	3	3	4	13	14	9	10	2	3	3	12	5
Oro	Oro	4	0	2	9	12	6	5	5	9	2	9	6
Oro	Hybrid 128	61	29	29	87	92	91	61	63	60	39	90	61
		T. levis, Race L–8											
Hybrid 128	Hybrid 128	74	55	71	76	94	91	77	86	71	67	87	78
Hybrid 128	Ridit	4	0	0.4	5	14	1	2	3	0	1	7	2
Ridit	Ridit	2	4	0	3	8	9	4	3	0	2	7	2
Ridit	Hybrid 128	70	49	53	91	95	91	74	80	69	57	92	74
Hybrid 128	Martin	0	0	0	0	2	0.4	1	0	0	0	1	0.3
Martin	Martin	0	0	0	0	0	0	0	0	0	0	0	0
Martin	Hybrid 128	24	45	62	86	97	97	59	55	81	43	93	65

TABLE 1.—(*Concluded*)

Wheat variety from which inoculum was taken	Wheat variety inoculated	Percentage of bunt											
		1935 Replication			1936 Replication			1937 Replication			Average		
		1	2	3	1	2	3	1	2	3	1935	1936	1937
		T. levis (Nonnumbered race)											
Hybrid 128	Hybrid 128	66	70	89	90	9	90	70	65	70	75	63	68
Hybrid 128	Ridit	4	6	0	9	5	13	9	15	2	3	9	9
Ridit	Ridit	0	7	0	2	0	3	5	2	4	2	2	3
Ridit	Hybrid 128	80	72	71	83	3	98	80	79	69	78	61	76
Hybrid 128	Oro	9	8	2	11	9	12	8	11	4	6	10	8
Oro	Oro	9	4	7	4	18	16	5	9	7	7	13	7
Oro	Hybrid 128	72	59	46	79	88	88	58	37	69	59	85	55
		Hybrid, *T. tritici* × *T. levis*											
Hybrid 128	Hybrid 128.	45	35	49	88	80	88	70	64	69	43	85	68
Hybrid 128	Turkey	0	4	1	0.3	3	0.4	3	1	1	2	1	2
Turkey	Turkey	5	11	10	15	11	7	41	43	20	9	11	35
Turkey	Hybrid 128	74	67	55	92	93	95	61	48	56	65	93	55

own bunt and inoculating it with the bunt from a susceptible variety. In addition, these cross inoculations contrast the difference in amount of infection on a susceptible variety when the inoculum was taken from a resistant variety and when it was taken from a susceptible variety.

RESULTS

The results from the 3 years' investigations are given in table 1. The percentages of smut infection differed from year to year, especially for the susceptible varieties, depending largely on environmental conditions. The amount of smut infection resulting from soil infestation in the uninoculated check rows ranged from 0.2 per cent to 3.5 per cent over the 3-year period.

The results presented in table 1 indicate that when a purified, physiologic race is used the amount of infection resulting from the reinoculation of a resistant variety with its own smut is not significantly higher than when inoculated with the same race from a susceptible variety. It is true that in some cases the percentage of infection increased as much as 4 or 5 per cent when a resistant variety was inoculated with its own smut; but this increase was not considered significant, since the increase was even greater in some instances when inoculum from a susceptible variety was put on a resistant one. For example, with race T–8 on Ridit, the average percentage of smut for 2 of the 3 years was the same and in the third year was 2 per cent higher when the inoculum was taken from the susceptible Hybrid 128 than when taken from Ridit. With race T–6 on Turkey the percentage of infection was higher each year when the inoculum was taken from Hybrid 128 than when taken from Turkey.

In 1936, *Tilletia tritici,* T–9 from Oro inoculated on Oro and T–10 from White Odessa inoculated on White Odessa were found, on microscopic examination, to have become accidentally mixed with some race or races of *T. levis* that were virulent on these two varieties. Therefore, the data for these two series are omitted for that year.

The results with the bunt species hybrid were somewhat different. When the inoculum was taken from the susceptible variety (Hybrid 128) and put on the resistant variety (Turkey) the percentage of infection failed to increase. However, when the inoculum was taken from the resistant variety, there was good evidence that straining was taking place. The segregates were being strained out in case of the reinoculation of the resistant variety and not in case of the susceptible variety.

SUMMARY

Six purified physiologic races of *Tilletia tritici,* 3 of *T. levis,* and a species hybrid were used in an experiment to determine the effect of repeated reinoculation of a resistant variety with its own smut on the percentage of infection obtained.

With the exception of the species hybrid, no significant increase in percentage of bunt infection was noted when resistant varieties were reinoculated with their own smut over a 3-year period.

In the case of the species hybrid there was an increase in infection each year on the variety Turkey, indicating that the inoculum to which this variety was resistant was being strained out, resulting in a concentration of the inoculum to which it was susceptible, thus resulting in higher percentages of infection.

The fact that purified physiologic races were used in these studies accounts for results differing from those of other investigators with the exception of Flor (2).

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TOMATO WILT RESISTANCE AND ITS DECREASE BY HETERODERA MARIONI

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Wilt caused by *Fusarium lycopersici* Sacc. seriously decreases yields of tomatoes (*Lycopersicon esculentum* Mill.) in East Texas. Hence, research was conducted to develop commercially desirable varieties of tomato resistant to *F. lycopersici.* Four varieties of X-ray-treated seed were tested to find desirable selections.

Tomato varieties resistant to *F. lycopersici* were developed and tested by Edgerton and Moreland (3), Norton (9), Pritchard (10), Huelsen (6), Lesley (7), Shapovalov and Lesley (11), and Wedgworth (13). Haymaker (5), White (14), and McWhorter and Parker (8) gave evidence of physiologic races of *F. lycopersici.* X-ray treatment of tomato seed was mentioned by Taubenhaus, Young, and Altstatt (12). Young and Taubenhaus (18) and Young (15, 16, 19) compared the wilt resistance of tomato varieties. Young (17) described methods of tomato culture. Altstatt and Young (1) reported longevity tests on *F. lycopersici.* Clayton (2) determined the relation of soil moisture to Fusarium wilt. Ficht (4) reported that *Heterodera marioni* decreased tomato yields. Young (20) stated that *H. marioni* contributes to the susceptibility of cotton to *Fusarium vasinfectum.*

ENVIRONMENT AND METHODS OF EXPERIMENTS

Tomato varieties were tested for wilt resistance in two nearly adjacent fields of 1.5 acres of Kirvin red soil abundantly infested with *Fusarium lycopersici.* This infestation was shown in 1936, when 99 per cent of the Gulf State Market tomatoes died of Fusarium wilt within 84 days after inoculation in one field, and 92 per cent of the Scarlet Dawn tomato plants died of wilt within 76 days after inoculation in the other field. In 1937, 95 per cent of 228 Stone tomato plants were killed by wilt, and the remaining plants showed wilt symptoms within 65 days after inoculation. Similarly, in 1938, 95 per cent of 130 Stone tomato plants died of wilt and the remainder showed wilt symptoms within 100 days after inoculation. All of the plants of many varieties died of Fusarium wilt in these fields. Although wilt killed the plants of several varieties more quickly in 1937 than in 1938, progress of the disease was quick and extensive enough both years to give comparable, accurate results in terms of time-weighted percentages of wilt resistance of the varieties tested.

For comparison with the plants in the red-soil fields, many varieties of tomato were planted in a sandy field (Norfolk fine sandy loam) of 2 acres having an extremely abundant infestation of *Fusarium lycopersici*. This sandy field also was abundantly infested with *Heterodera marioni* (Cornu) Goodey. In 3 years, this field was tested with 4710 watermelon plants, 87.6 per cent of which showed root-knots caused by *H. marioni*. Tomatoes in this field, likewise, were damaged by root-knot.

The soil of the 3 fields was slightly acid (pH 6.5). Favorable soil moisture and temperature prevailed often and long enough to facilitate rapid and extensive development of Fusarium wilt. In hot weather, such favorable temperature occurs at night and in rainy periods. Droughts occurred from May 24 to June 3 and from June 13 to July 9, 1937; also from July 6 to 17, 1938. Amounts of water favoring *Fusarium lycopersici* presumably occurred as the soil dried after saturation by rains. The annual rainfall of this region is approximately 41 in. Water adequate for good growth of the tomato plants apparently was adequate for infection and killing of the plants by *F. lycopersici*.

The following methods were used in raising the tomatoes. The soil of the hot beds and cold frames was disinfected with chloropicrin injected at the rates of 300 to 600 lb. per acre. Tomato seed was treated with Cuprocide and planted in the flue-heated, cloth-covered hot beds in the first 10 days of February. The seedlings were transplanted into cold frames in the first week of March. Nearly all of the tomato plants were healthy and were inoculated by transplanting them into the field soil infested with parasites, in the first 10 days of April. The plants were set 21 in. apart in rows 5 ft. apart on beds 8 in. high. The soil was fertilized with 6–10–7 fertilizer at the rate of 600 lb. per acre, and was cultivated every week to keep the soil crust broken and to control the weeds. Each tomato plant was pruned to one main stem and tied to a stick. The plants were sprayed with copper compounds and arsenates, which prevented insects and diseases of leaves and fruits from interfering with the experiments. The plants in the yield test were sprayed identically. Diseases of the leaves and fruits were rare in 1937 and 1938. Tomato yields were calculated with the formula: Pounds of fruit per plant times the ton constant (which is 1 2000 of the number of plants per acre) equals tons of fruit per acre.

OLD METHOD OF DETERMINING WILT RESISTANCE

The degrees of wilt resistance of 4 tomato varieties were compared by determining the percentages of plants with wilt symptoms on June 9 and 25, respectively, and the plants still green on June 25, 1936. This old method sufficed merely to show that Marglobe was resistant, Gulf State Market was susceptible, and Scarlet Dawn was very susceptible to Fusarium wilt without clearly showing degrees of resistance or susceptibility. For additional information, late survival of plants also was determined on July 16, 1937. The following varieties were the only ones that showed as many as 25 to 40 per cent of their plants surviving the disease as late as 2 weeks after the end of the shipping season: Blair Forcing, Buckeye State, Kanora, Louisiana Pink, Louisiana Red, Marglobe, Marvana, Marvel, Michigan State, Norduke, Rutgers, and Sureset Forcing. These varieties showed similar late survival in 1938 as evidence of strong resistance to Fusarium wilt.

Nearly all of the plants of the other 71 varieties died of wilt within 100 days after inoculation with *Fusarium lycopersici* in the red-soil fields in 1937 and 1938; so the old simple percentage method was inadequate in separating the varieties clearly on the basis of wilt resistance. No simple, easy method proved satisfactory for this purpose, especially for commercially valuable wilt resistance.

TIME-WEIGHTING METHOD USED IN COMPARING WILT RESISTANCE OF TOMATO VARIETIES

Economically profitable wilt resistance is complicated especially with time, as the earlier the plants wilt the less they yield. As soon as the leaves show wilt symptoms, the plant is diseased badly enough to decrease its yielding ability, even though it continues to live for a few weeks. The commercial shipping season in east Texas usually is May 24 to July 1. Hence, plants wilting by June 1 represent much greater commercial loss than do those that are alive in July despite wilt symptoms. It was thus necessary to use time as a main factor in deriving an algebraic formula to separate more than 200 selections on the basis of their economic wilt resistance. The following formula was derived and used in calculating the time-weighted percentages of wilt resistance of tomato varieties in 1937 and 1938 (Tables 1, 2, 3).

$100 \text{ per cent} - (.2a + .2b + .15c + .2d + .e + .f + .05g) = h$, where

a is the percentage of plants with wilt symptoms nearly 100 days after seedlings emerged,
b " " " " " wilt-killed " 112 " " " "
c " " " " " with wilt symptoms " 112 " " " "
d " " " " " wilt-killed " 125 " " " "
e " " " " " with wilt symptoms " 125 " " " "
f " " " " " wilt-killed " 145 " " " "
g " " " " " with wilt symptoms " 145 " " " "

h is the time-weighted percentage of wilt resistance of the tomato selection to *Fusarium lycopersici*. The dates for records were selected for early tomatoes emerging about Feb. 10 in cool soil and producing green-wrap fruit in nearly 100 days.

TABLE 1.—*Tomato-wilt resistance decreased by Heterodera marioni*

Tomato variety	T. No.	No. of plants	Time-weighted percentage of wilt resistance		
			Soil without *H. marioni*	Soil with *H. marioni*	Decrease by *H. marioni*
Early Baltimore; W. A. Huelsen	42^{a}	116	61	53	8
Early Detroit; W. Atlee Burpee Co.	257	170	33	16	17
Grothen Red Globe; Landreth Seed Co.	27^{a}	165	67	33	34
Guba, E. F.; *L. pimpinellifolium* cross	195	69	70	57	13
Gulf State Market; 8 selections	451^{b}	966	55	37	18
Kanora; Barteldes Seed Co.	48^{a}	142	80	58	22
Landreth Globe; Landreth Certified	26^{a}	165	47	35	12
Livingston Globe; Asgrow	16^{a}	192	54	31	23
Lloyd Forcing; W. A. Huelsen	46^{a}	211	61	37	24
Louisiana 10–4; Landreth Seed Co.	84	91	74	43	31
Louisiana Gulf State; Reuter Seed Co.	127	67	54	43	11
Louisiana Pink; Haven Seed Co.	35^{a}	166	83	73	10
Louisiana Red; Haven Seed Co.	34^{b}	392	86	72	14
Marglobe; 4 selections	449^{b}	228	88	68	20
Marhio; Associated Seed Growers	10^{a}	233	70	47	23
Marvana; 2 selections	450	251	64	58	6
Marvel; Haven Seed Co.	37^{a}	181	81	69	12
Marvelosa; W. S. Porte	57^{a}	133	67	48	19
Michigan State; Vaughan Seed Store	51^{a}	97	78	61	17
Norton; Associated Seed Growers	13^{a}	224	35	25	10
Prairiana; W. A. Huelsen	40^{a}	175	71	58	13
Redfield Beauty; Hastings	28	59	72	46	26
Riverside; M. Shapovalov	72	66	83	75	8
Rutgers; L. G. Schermerhorn	7^{a}	217	78	39	39
Stone; Associated Seed Growers	14^{b}	423	22	16	6
Sureset Forcing; W. A. Huelsen	45^{a}	216	76	57	19
Urbana Forcing; W. A. Huelsen	47^{a}	164	61	38	23

[a] Test in 1937; [b] test in 1937 and 1938; all other tests in 1938.

Two varieties of tomato, growing in the red-soil fields in 1938, are used as examples of the calculation of the time-weighting formula. In this field, a completely susceptible variety would have all of its plants killed by wilt within 50 days after inoculation, while a completely resistant variety would not show wilt symptoms in any of its plants within 96 days after inoculation by setting cold-frame plants in fields. Although no tomato varieties were found to illustrate these extremes, the two following varieties differed 72.2 per cent in their time-weighted percentages of wilt resistance, and exemplify the method of calculation:

a	48.8	per cent of plants	wilted	by May 24	× .2	equals	9.76	per cent susceptible.
b	61.6	" " " "	wilt-killed	by June 3	× .2	"	12.32	" " "
c	98.4	" " " "	wilted	by June 3	× .15	"	14.76	" " "
d	99.2	" " " "	wilt-killed	by June 18	× .2	"	19.84	" " "
e	100.0	" " " "	wilted	by June 18	× .1	"	10.0	" " "
f	100.0	" " " "	wilt-killed	by July 9	× .1	"	10.0	" " "
g	100.0	" " " "	wilted	by July 9	× .05	"	5.0	" " "

Total equals 81.68 per cent susceptible.

TABLE 2.—*Time-weighted percentages of wilt resistance of tomato varieties*

Tomato variety	No. of plants	Wilt resistance	Tomato variety	No. of plants	Wilt resistance
Baltimore; T452	230	67	Long Calyx Forcing; T44[a]	238	65
Blair Forcing; T43[a]	201	76	Louisiana Dixie; T126	62	73
Break O'Day; T23[a]	159	55	Louisiana Pink; T458[b]	379	86
Brimmer; T83	63	68	Louisiana Red; T459	103	90
Browns Special; T86	47	38	Marglobe; T457[b] (11 sel.)	834	76
Buckeye State; T39[a]	222	80	Marhio; T10	68	80
Century; T453	70	80	Marvana; T56[a]	58	80
Clarks Special B; T6[a]	78	54	Marvelosa; T50[a]	125	46
Columbia; T38[a]	214	30	Newport 5; T194	163	77
Early Baltimore; T177	65	71	Norduke; T52[a]	81	84
Everbearing Scarlet Globe	72	61	Nystate; T49[a]	95	36
Globelle 1; T229	67	72	Pritchard; T22[a]	183	60
Golden Queen; T228	92	53	Red Cherry; T225	66	51
Greater Baltimore; T25[a]	153	35	Red Rock; T455	171	19
Grothen Red Globe; T90	41	77	Rutgers; T456[b]	557	78
Gulf State Market; T454[b]	271	52	Summerset; T260	24	57
Illinois Baltimore; T157	68	91	Sweetmeat; T239	47	67
Illinois Pride; T41[a]	208	65	Tennessee Red; T235	28	81
Indiana Baltimore; T208	20	64	Wenholz cross; T431	139	63
John Baer; T256	138	33	Wenholz cross; T251	25	10
Landreth; T30	62	35	Yellow Ponderosa; T227	36	72

[a] Test in 1937; [b] test in 1937 and 1938; all other tests in 1938.

TABLE 3.—*Wilt resistance affecting tomato yields in an epiphytotic of wilt caused by Fusarium lycopersici*

T. No.	Tomato variety	No. of plants	Marketed yield of fruit in tons per acre	Weighted percentage of wilt resistance	Blossom-end rot; no. of fruits per plant
43	Blair Forcing; Vaughan's Seed Store	132	3.68	84	.1
93	Break O'Day; Landreth Seed Co., Certified	137	5.15	72	.7
257	Early Detroit; W. Atlee Burpee Co.	135	0.61	33	1.3
129	Gulf State Market; Landreth; Crown Picked	137	3.53	48	1.1
94	Hastings Extra Early Prolific	138	3.29	58	1.0
11	Louisiana Pink; Associated Seed Growers	129	4.85	90	.7
34	Louisiana Red; Haven Seed Co.	138	4.08	89	1.6
87	Marglobe; Landreth Seed Co., Certified	121	4.71	81	.3
63	Marglobe; YH selection from Reuter Marglobe	131	4.83	79	.6
156	Michigan State; Vaughan's Seed Store	125	5.25	78	.4
158	Prairiana; Vaughan's Seed Store	128	3.83	73	2.9
92	Pritchard; Landreth Seed Co., Certified	132	5.40	74	.16
89	Rutgers; Landreth Seed Co., Certified	136	4.98	82	2.1
14	Stone; Associated Seed Growers (Asgrow)	130	0.20	26	1.4

Subtracting this 81.68 per cent of susceptibility from 100 per cent leaves 18.32 (h) time-weighted percentage of resistance of T433 Red Rock (125 plants) to *F. lycopersici.*

a	0	per cent of plants	wilted	by May 24	× .2	equals	0	per cent susceptible.
b	0	" " " "	wilt-killed	by June 3	× .2	"	0	" " "
c	2.2	" " " "	wilted	by June 3	× .15		.33	" "
d	0	" " " "	wilt-killed	by June 18	× .2		0	" "
e	33.7	" " " "	wilted	by June 18	× .1		3.37	" "
f	15.7	" " " "	wilt-killed	by July 9	× .1		1.57	" "
g	84.3	" " " "	wilted	by July 9	× .05		4.21	" "
					Total	"	9.48	" " "

h equals 9.48 per cent of susceptibility, subtracted from 100 per cent leaves 90.52 time-weighted percentage of resistance of T432 Louisiana Pink (89 plants) against *F. lycopersici.*

WILT RESISTANCE OF TOMATO SELECTIONS

Data showing the wilt resistance of many tomato varieties are summarized in tables 1 and 2. Each line in the table represents 1 to 9 plots of 18 or more plants per plot distributed at random in the fields, so that wilt-resistant varieties were near susceptible ones. "T" is the serial number of each selection or seed stock of tomatoes.

The time-weighted percentages of wilt resistance were 6 to 39 per cent lower for 47 of the 54 tomato selections tested in the sandy field than for these varieties tested in the red-soil fields, because the tomatoes in the red-soil fields were infected only with *Fusarium lycopersici,* while most of those in the sandy field were infected also with *Heterodera marioni.* Four other selections were 0 to 5 per cent less wilt-resistant in the sandy field than in the red-soil fields, while 3 wilt-susceptible varieties showed 5 to 10 per cent more wilt resistance in the sandy field than in the red-soil fields (Table 1). It is not surprising that the infection of the roots with *H. marioni* decreased their vitality and increased their susceptibility to *F. lycopersici* in the sandy field in contrast to the red-soil fields, where *H. marioni* did not occur. The wilt resistance was decreased by root knot more in some tomato varieties than in others, probably because of differences in the severity of root knot. Wilt resistance probably was too low in the 3 exceptional varieties to be decreased by root knot.

It is necessary to have fields with almost maximum infestation of *Fusarium lycopersici* for accurately testing tomatoes for varietal resistance to wilt. However, it is possible to prove that a field has a little less than maximum infestation of *F. lycopersici* only by comparing it with a neighboring field proved to have essentially maximum infestation. Extensive tests in 1937 and 1938 showed that the 2 red-soil fields considered in the tables had almost the maximum infestation. One of these fields (West-half) was adjacent to a nearly identical field (East-half), tested in comparison in 1936 and 1938. In tests recorded for thousands of plants on June 25, 1936, Marglobe plants in the East-half field had only 34 per cent of the plants wilted, while in the West-half field, Glovel showed 75 per cent wilted, and Gulf State Market, 91 per cent wilted. There was no crop in the East-half field, while tomatoes were tested in the West-half field in 1937. Twenty-four varieties, including 51 selections, were grown in both the East-half and West-half fields in 1938. Most of the plants wilted a little later in the East-half than in the West-half field, although by July 20, the disease had killed nearly all of the plants in both fields. Because of this delay, 40 of the 51 selections had time-weighted percentages of wilt resistance 6 to 41 (mostly near 20) per cent higher in the East-half than in the West-half field. Seven other selections had such percentages of wilt resistance 0 to 5 per cent higher, but 4 other selections had such percentages of resistance 1 to 10 per cent lower in the East-half than in the West-half field. This evidence shows that most parts of the East-half field had a little less than maximum infestation before July; so data from this field were excluded from tables 1 and 2.

Table 3 compares the yields of 14 tomato varieties in the West-half field having virtually maximum infestation of *Fusarium lycopersici*. Each variety was planted in 3 units distributed at random on ½ acre of land. The yields are given in comparison with the time-weighted percentages of wilt resistance and the numbers of fruits discarded because they had blossom-end rot. In this field, other tests with 40 or more plants per variety gave the following yields in tons of marketable fruit per acre: Illinois Baltimore 4.0, Kanora 3.0, Louisiana Dixie 2.2, Louisiana Gulf State 3.4, T125 Marglobe 4.7, T194 Newport 5.3, and Sweetmeat 4.2 tons.[1]

Blair Forcing (T43) and Lloyd Forcing (T46), related varieties of greenhouse tomatoes, developed prominent upward rolling of most of their leaflets in hot dry weather in fields. The leaflets remained rolled in following wet weather. This apparently is a physiologic type of leaf roll.

P.I.117566 from Brazil had red, vertically wrinkled fruits, 1 to 2 in. long and 1 in. in diameter. The fruits were completely hollow, except for the seed mass, thus resembling pepper fruits. This "Hollow" kind of tomato is a new variety that is morphologically distinguished by its hollow fruits. It was wilt-resistant.

Large differences were found in the wilt resistance of separate selections of tomatoes. The following time-weighted percentages of wilt resistance exemplify the range in resistance of different selections in fields extremely infested with *F. lycopersici*, but practically free from *H. marioni*. Eight selections of Baltimore: 58 to 74 per cent; 4 selections of Century: 40 to 85 per cent; 18 selections of Gulf State Market: 46 to 69 per cent; 5 selections of Louisiana Pink: 83 to 91 per cent; 4 selections of Louisiana Red: 83 to 93 per cent; 31 selections of Marglobe: 25 to 91 per cent; 13 selections of Rutgers: 66 to 87 per cent; and 3 selections of Summerset: 30 to 57 per cent. It is thus important to determine locally the wilt resistance of each seed stock or selection of commercially valuable varieties.

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[1] After this article was written, information on tomato yields and a method of grading wilt resistance was found in the following article: Cook, H. T. 1938 tomato-seed-source demonstration in Virginia, U. S. D. A. Extension Pathologist 36: 22–25. 1939 (Mimeographed).

SUMMARY

The time-weighted percentages of wilt resistance of 83 varieties including 207 selections of tomatoes were determined by growing their plants in epiphytotics of wilt caused by *Fusarium lycopersici* in the practical absence of other tomato parasites. These percentages were calculated with a new formula that was derived to time-weight 7 percentages of wilting and wilt-killed plants. Time-weighted wilt resistance of 70 per cent or more is needed in commercially desirable varieties of tomatoes.

These time-weighted percentages describe with mathematical precision the wilt resistance of the tomato varieties under the environmental conditions described. However, according to the general rule, the wilt resistance of tomato varieties is expected to vary in different environments that cause differences in details of cell physiology and structure of both host and parasite. Different physiologic races of the parasite also affect wilt resistance. Scarlet Dawn, Glovel, and Norton varieties are markedly susceptible to *Fusarium lycopersici* in this region, although they are described as being wilt-resistant elsewhere.

Selections of Blair Forcing, Buckeye State, Illinois Baltimore, Louisiana Pink, Louisiana Red, Marglobe, Riverside, and Rutgers showed the most marked wilt resistance of the varieties of tomatoes tested. Of the 14 varieties in a special yield test, Pritchard, Michigan State, Break O'Day, Rutgers, Louisiana Pink, and Marglobe gave the best yields in an epiphytotic of Fusarium wilt. Large yields were directly correlated with marked wilt resistance.

Extensive evidence showed that *Heterodera marioni* greatly decreased the time-weighted percentages of resistance of many tomato varieties to *Fusarium lycopersici*.

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EFFECT OF SULPHURIC-ACID TREATMENT ON FUNGI AND BACTERIA PRESENT ON COTTON SEED FROM DISEASED BOLLS[1]

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(Accepted for publication April 20, 1939)

Delinting of cotton seeds with sulphuric acid has been advocated for control of seed-borne diseases, for quicker germination, and for improvement in the stand of plants. Considerable field work and some laboratory work has been done in this connection by various investigators. This paper presents the results of laboratory study involving the culture of fungi and bacteria from acid-delinted seeds with reference to the effect of length of treatment on the viability of seed-borne organisms and on germination of the seed. Studies also were made of gravity-separated, delinted seeds and the presence of fungi and bacteria upon them.

Several writers (Duggar and Cauthen (4), Rolfs (8), Faulwetter (6), Elliott (5), Archibald (1), Stoughton (9), and Laycock (7)) have dealt with the delinting of cotton seeds in sulphuric acid for the control of seed-borne organisms such as *Bacterium malvacearum* E. F. S. and *Glomerella gossypii* (E. A. Southw.) Edg. In general, results have shown that treatment in acid reduces the percentage of disease caused by these organisms. Crawford (3) isolated *Fusarium vasinfectum* Atk., *Fusarium* spp., *Colletotrichum, Diplodia, Alternaria,* and *Cephalothecium* from acid-treated seeds of wilt-infected plants. As to gravity separation, work has been reported by Webber and Boykin (12), Taubenhaus and Kernkamp (11), Taubenhaus and Burkett (10), Chester (2), and others. Field plantings of these gravity-separated seeds indicate that sinking seeds produce better stands of cotton. About 40 per cent of the seeds tested by Chester were of the floating type, and in germination tests about 50 per cent of the floating seeds were viable as compared with 99 to 100 per cent germination for the sinking seeds.

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[1] This work was carried out at the Texas Agricultural Experiment Station from April 1936 to September 1937, while the author held a fellowship granted by the Freeport Sulphur Company in the Division of Plant Pathology and Physiology. The assistance of the late Dr. J. J. Taubenhaus in outlining the problem is gratefully acknowledged.

Approved by the Director as Contribution No. 521, Technical Series, of the Texas Agricultural Experiment Station.

During August and September, 1936, several hundred bolls were collected in a field at Bryan, Texas, from cotton plants heavily infected with *B. malvacearum*. All of these bolls had typical boll-rot lesions caused by this organism. The cotton in each boll was ginned separately in a small electric gin, and the seeds stored in separate packets Four groups totalling approximately 2000 seeds each were treated with concentrated sulphuric acid for 15, 30, 45, and 60 minutes, respectively. After treatment, the acid was drained off and the seeds were rinsed three times in sterile distilled water. At this point the sinking seeds were separated from those that floated in the distilled water The seeds were then plated on potato-dextrose agar in Petri dishes (Fig 1). Five seeds or less were placed in a

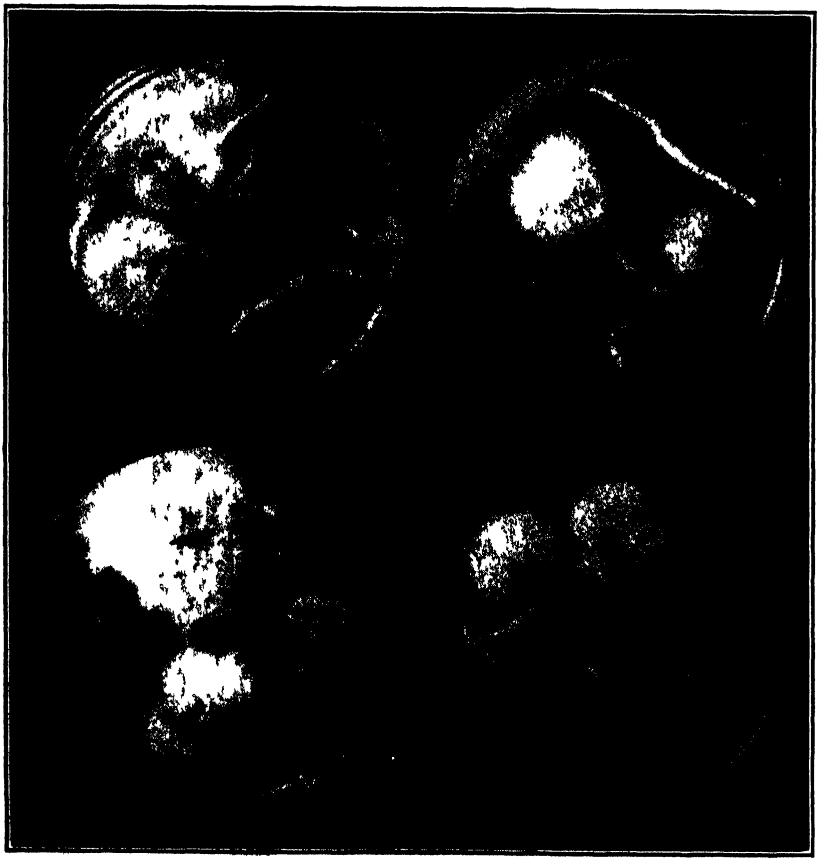

FIG. 1. Cultures showing method of ascertaining the presence of seed borne organisms and germination of acid-delinted cotton seed.

TABLE 1.—*Organisms recovered from cotton seeds delinted in acid and divided into floating and sinking lots*

Organisms recovered[a]	Percentage of seeds from which organisms were recovered following treatments for periods indicated								
	15 minutes		30 minutes		45 minutes		60 minutes		Av. for all treatments
	Floating (659)[b]	Sinking (142)	Floating (626)	Sinking (92)	Floating (753)	Sinking (158)	Floating (712)	Sinking (229)	
Sordaria	10.9	1.4	19.8	9.8	10.5	1.8	1.8	0.3	8.8
Diplodia	10.3	1.4	4.1	3.2	5.4	1.8	1.8	0.8	4.7
Fusarium	7.4	5.6	2.2	0.0	1.8	2.5	2.4	3.1	3.1
Sterile mycelium	0.4	0.0	5.6	7.6	3.4	2.5	0.7	0.8	2.4
Alternaria	2.0	1.4	1.3	0.0	1.2	0.0	4.2	3.0	2.2
Sclerotium	3.0	0.7	0.6	2.1	3.0	1.8	0.1	0.0	1.6
Unidentified	0.3	1.4	0.0	0.0	2.2	1.8	1.9	2.1	1.2
Bacteria[c]	2.3	0.0	3.2	0.0	0.1	0.0	0.1	0.0	1.1
Basisporium	0.0	0.0	0.0	0.0	0.5	0.0	1.1	5.6	0.7
Gloeosporium	0.0	0.0	0.2	4.3	0.9	0.0	0.0	0.0	0.3
Phoma	0.4	0.0	0.6	0.0	0.0	0.0	0.1	0.4	0.3
All organisms	37.0	11.9	37.6	27.0	29.0	12.2	14.2	16.1	26.4

[a] In some cases more than one colony of the same or different fungus was isolated from one seed.

[b] Number of seeds in this group.

[c] Cultural characteristics of these bacteria resembled closely those of *B. malvacearum*, but inoculation experiments made 4 months after isolation of the organism produced no symptoms of angular leaf spot.

Petri dish, and after 2-day intervals the plates were examined for bacterial or fungous growth. Percentage of germination was taken on the fourth day (few seeds germinated after this period). Sub-cultures of any fungi or bacteria that had originated from the seeds were made on the sixth day. All isolates were grown on slants of potato-dextrose agar. Because of the variety and abundance of organisms obtained from culturing fuzzy seeds, no cultural studies were made before delinting.

All fungi that appeared to belong to the genus *Fusarium* were sent to C. D. Sherbakoff at the Tennessee Agricultural Experiment Station for identification. Readily distinguishable organisms were identified by the writer; other cultures of unknown fungi were sent to the Bureau of Plant Industry, Washington, D. C., to be identified.

Although bacteria and fungi were obtained after all 4 treatments in sulphuric acid (15, 30, 45, and 60 minutes), the percentage of seeds from which fungi were recovered varied only slightly (Table 1) for the 15- and 30-minute periods. With the longer treatments (45 and 60 minutes), however, fewer fungi were recovered. It will be noted also that prolonged treatment in acid reduced the number of colonies of bacteria obtained from the seeds. Although only those colonies of bacteria that superficially resembled *Bacterium malvacearum* were considered, inoculation trials with these cultures 4 months after isolation of the organisms gave no leaf-spot infection. Among the fungi, the most common genera listed in order of their prevalence were *Sordaria, Diplodia, Fusarium, Alternaria,* and *Sclerotium*. Certain Fusaria were identified as *F. moniliforme* Shel., *F. bullatum* Sherb., *F. bullatum* var. *roseum, F. semitectum* B. and Rav., and *F. oxysporum* Schl. The species of *Diplodia* was *D. natalensis* Evans.

There was a marked difference between the percentage of sinking and floating seeds that yielded organisms in culture. For all of the treatments, the floating seeds showed the highest percentage of infection. No particular fungus was more common on floating seeds than on sinking seeds. It should be noted here that the percentage of floating seeds obtained in this work was considerably higher than that reported by Chester (2).

Results obtained from germination tests of cotton seeds that received the acid treatment of various lengths and then separated by floating are shown in table 2. Nearly as many fungi were isolated from germinated seeds as from those that did not germinate.

TABLE 2.—*Effects of different acid treatments on germination of sinking and floating seeds of cotton*

Length of treatment	Percentage of seeds floating	Percentage germination	
		Floating	Sinking
15 min.	82	61.0	90.8
30 "	87	71.5	82.5
45 "	83	75.0	93.6
60 "	75	86.9	93.4
Av.	81.7	73.8	90.0

An additional treatment of about one-half (4000) of the delinted seeds in alcoholic mercuric chloride solution (1–1000) apparently had little effect on the number or type of fungi or bacteria obtained in culture as compared with the acid treatment alone. These seeds also germinated as readily (floating 74 per cent, sinking 89 per cent, average of 4 treatment periods) as those which received the acid treatment only.

After fungi and bacteria had been isolated from acid-treated seeds, culture work was planned to demonstrate whether such organisms are on the outer or inner parts of the seed. The seed coats of nearly 400 delinted (60-minute treatment) seeds were separated from the embryos aseptically and these parts were cultured separately. Five colonies of *B. malvacearum* were recovered from 4 separate seeds, 3 from seed coats and 2 from embryos. All of these cultures produced definite angular leaf-spot lesions when suspensions of the organisms were sprayed immediately on young cotton plants. Certain fungi were recovered also from these cultures; *Sordaria* being the most common. In the limited number of trials, the seed coats yielded more fungi than the inner parts of the seeds. In one particular boll, 16 of the 18 seeds yielded *Rhizopus* from both the seed coat and the embryo. These results indicate that a small percentage of fungi and bacteria may become established within the seed, and suggest that surface treatment of seeds, as by sulphuric acid, may not eliminate this final small percentage of internal infection.

SUMMARY

Nearly 8000 cotton seeds from bolls infected with *Bacterium malvacearum* were divided into 4 groups and treated with concentrated commercial sulphuric acid for periods of 15, 30, 45, and 60 minutes, respectively. Approximately one-half of these seeds were given an additional treatment in a solution of mercuric chloride in 50 per cent alcohol (1–1000). The seeds were cultured in Petri dishes on potato-dextrose agar.

With the longer acid treatments, fewer fungi and bacteria were obtained in culture. The additional mercuric chloride treatment had no marked effect upon the number of seed-borne organisms obtained in culture nor upon germination.

Fewer fungi and bacteria were recovered from sinking seeds than from floating seeds, and sinking seeds gave the highest percentage of germination.

In a special test with 400 seeds, which had been treated in acid for 60 minutes and then dissected aseptically, 5 colonies of *B. malvacearum* were recovered—3 from the seed coat and 2 from the embryo.

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INJURY TO TOBACCO LEAVES BY WATER-SOAKING[1]

W. D. VALLEAU, STEPHEN DIACHUN, AND E. M. JOHNSON

(Accepted for publication April 17, 1939)

It is claimed that water-soaking of leaf tissues is necessary for the development of "epidemic" wildfire and late-season blackfire of tobacco.[2, 3] Clayton believes that water-soaking breaks down the resistance of maturing tobacco leaves to the bacteria. He reports[2] that "for the development of the destructive type of wildfire the water-soaked areas must persist for 24 hours or more after inoculation has occurred," while the water-soaked condition must persist 48 hours for the development of blackfire.[3] He claims that water-soaked leaves recovered without injury when not inoculated, but does not state whether the noninoculated leaves were kept water-soaked for the 24- or 48-hour period, or were water-soaked and then returned to the greenhouse where the water would quickly disappear, leaving no injury. The possibility that asphyxiation of individual cells might occur when they were surrounded by water for 24 or 48 hours in the absence of invading organisms apparently was not given consideration.

J. Johnson[4] reports that plants were water-soaked by application of a high water pressure to the root system or the cut stem. When they were kept in a saturated atmosphere, where the water-soaked condition could be maintained for several days if desired, inoculated plants usually developed symptoms after 8 to 48 hours in the moist chamber. If such water-soaked plants were taken out of the apparatus without inoculation and the roots or cut end of the stem were placed in water at atmospheric pressure, transpiration at the lower humidities rapidly removed the excess water in the leaves. "The uninoculated recovered plants, even though kept under water pressure for 12 hours, show no sign of any physical internal or external injury to the tissues." He reported that the inoculated plants were usually left in the saturated humidity chamber until they showed signs of infection, usually from 24 to 72 hours.

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[1] The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station, and is published by permission of the Director.

[2] Clayton, E. E. Water soaking of leaves in relation to development of the wildfire disease of tobacco. Jour. Agr. Res. [U. S.] 52: 239–269, 1936.

[3] Clayton, E. E. Water soaking of leaves in relation to development of the blackfire disease of tobacco. Jour. Agr. Res. [U. S.] 55: 883–889, 1937.

[4] Johnson, J. Relation of water soaked tissues to infection by *Bacterium angulatum* and *Bact. tabacum* and other organisms. Jour. Agr. Res. [U. S.] 55: 599–618, 1937.

From the published reports of these two workers it is difficult to tell whether water-soaked noninoculated leaves were ever kept in the water-soaked condition for 24 hours or longer. It is the purpose of this paper to report injury caused by water-soaking alone, without inoculation.

METHOD

Because early attempts to water-soak leaves in the manner described by Clayton frequently produced such a high degree of permanent injury, a simple apparatus was arranged that could be used to water-soak leaves with or without injury, at will. A nozzle consisting of a glass capillary tube 2 cm. long with a .75 mm. bore was inserted into one end of a heavy-walled rubber tube of convenient length, attached to a ⅛ in. iron pipe fitted with a pressure gauge and valve; the pipe was joined by proper fittings to a garden hose that connected with the water line. The pipe with valve and gauge was mounted on a ring stand. The water pressure in the rubber tube was regulated by the gauge and valve. With a pressure of 7½ lb. per square inch the portion of the stream of water nearest the nozzle appears to be more or less a solid column; at a point 10 to 12 cm. from the nozzle the solid column gradually breaks up into a spray of droplets surrounding a central more or less continuous column which gradually spreads out into a spray of drops.

If the end of the nozzle was pressed against the lower surface of a leaf an area limited by veins became water-soaked. When the nozzle was held any distance from the leaf up to the breaking point of the stream the leaf became water-soaked rather slowly. It was not always possible to water-soak leaves with the solid column. If the nozzle was held 10 to 12 cm. from a susceptible leaf, at the point where the column of water was being disrupted, water soaking could be produced rapidly if the nozzle was moved as fast as tissue became soaked. If the nozzle was held more than 12 to 15 cm. from the leaf water-soaking occurred more slowly the farther the nozzle was removed.

The water-soaked condition could be maintained only when the plants were kept in a fog or mist formed by several spray nozzles surrounding the plant.

These studies were made on White Burley tobacco variety Kentucky No. 16, grown in half-gallon jars or in a ground bench in the greenhouse.

MECHANICAL INJURY PRODUCED BY WATER-SOAKING

In this report a distinction is made between mechanical and physiological injury to water-soaked leaves.

The amount of water pressure, the distance of the nozzle from the leaf surface, and the duration of spraying determined whether or not mechanical injury would result from water-soaking. Usually there was no mechanical injury when the nozzle was held about 30 cm. from the leaf with 5 to 8 lb. pressure per sq. in. When the leaf was placed at the breaking point of the column or a little beyond with pressure of 10 lb. or more, injury resulted from the beating of water. Under these conditions, even if the excess water was lost within as short a time as 30 minutes, the lower surface soon became slightly roughened or pebbly. After 3 or 4 days some of the tissue became chlorotic to necrotic and dried out in spots (Fig. 1). The injury seemed to occur more often on leaves that water-soaked slowly, probably because they were subjected to a longer period of bombardment. This type of injury resembled the spotting of leaves known locally as "moonburn," which often occurs in the field after wind-and-rain storms and in the absence of infection. Figure 1 shows moonburn, characterized by the pebbly appearance of the lower surface and chlorotic indistinct spots with necrotic centers on the upper surface, which were free from pathogenic bacteria.

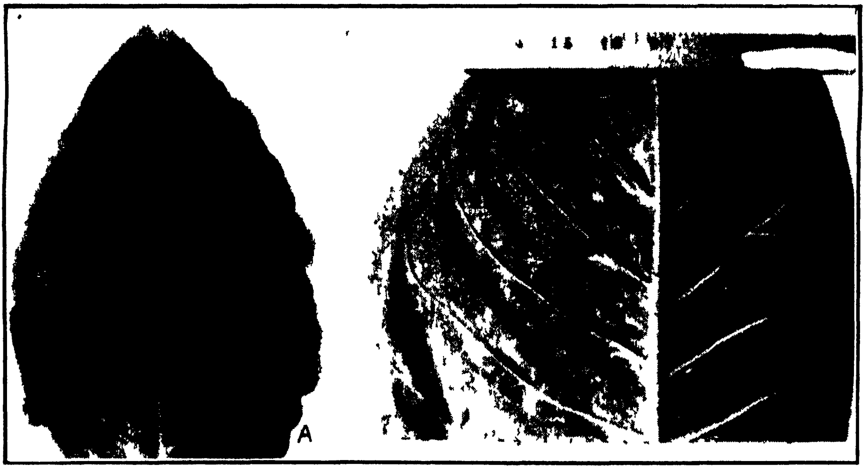

FIG. 1. A. Mechanical injury on the upper left side of leaf resulting from beating of water on the lower surface; the lower surface is slightly roughened or pebbly; the leaf is chlorotic to necrotic in spots. B. Moonburn on the lower surface of a leaf of a field plant following a wind-and-rain storm; the chlorotic and necrotic spots were free from infection.

PHYSIOLOGICAL INJURY

When leaves were water-soaked with the nozzle held against the lower leaf surface or a short distance away (up to 6 cm.), or with the nozzle 30 cm. or more from the leaf, and then allowed to lose the excess water at once, there was no apparent injury to water-soaked areas. This is in accord with the results of Clayton. However, if leaves water-soaked in any of these ways were kept water-soaked 48 hours and then allowed to lose the excess water, they sometimes began to show signs of injury a day or two later. At first the water-soaked areas were apparently healthy, but they gradually

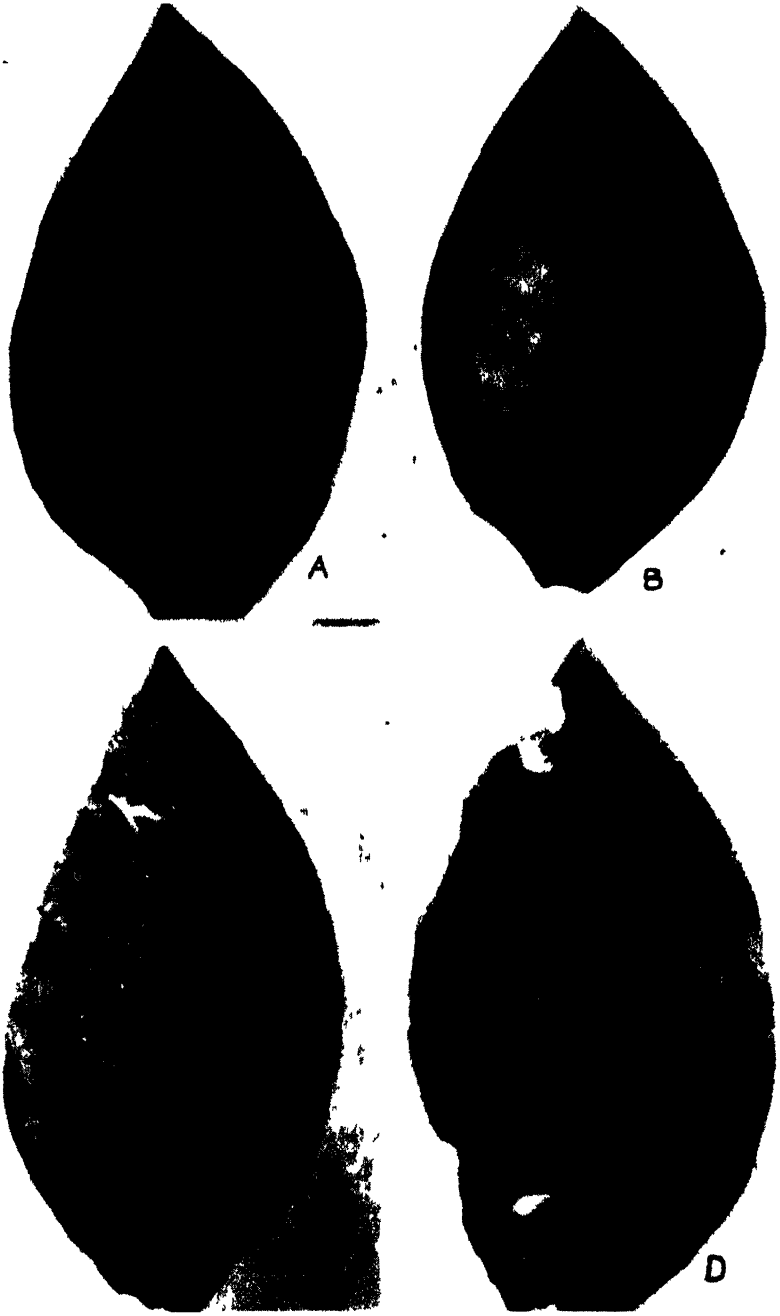

FIG. 2. The left side of each leaf was water-soaked after removal from the plant. A. Allowed to lose excess water at once; the leaf is normal. B. The tissues kept water-soaked 24 hours are chlorotic. C. The tissues kept water-soaked 48 hours are chlorotic and becoming necrotic in spots. D. The tissues kept water-soaked 72 hours are necrotic. Photographed 6 days after the leaves were water-soaked.

became chlorotic and often severely necrotic and brown. Injury of this nature could be produced on detached water-soaked leaves more readily than on attached leaves. On June 21, 1938, one-half of each of 8 middle leaves of plants in a ground bench was water-soaked following removal from the plant, with the nozzle held 30 cm. from the lower leaf surface and a pressure of 7½ lb. per sq. in. in the line. Two leaves were then placed in the laboratory with the ends of the petioles in a beaker of water. The rest were kept in the greenhouse in a fine spray of water; 2 of these were removed from the spray, and placed in a beaker of water in the laboratory 24 hours after water soaking; 2 after 48 hours, and 2 after 72 hours. The water-soaked condition was maintained as long as the leaves were kept in the spray. The excess water was lost about 2 hours after the leaves were removed from the spray.

Two days after the last leaves were removed from the moist atmosphere, the areas kept water-soaked 24 hours were chlorotic; those water-soaked 48 hours were chlorotic with necrotic brown areas, and those kept water-soaked 72 hours were brown and necrotic. The areas on the leaves water-soaked and allowed to lose the excess water at once, however, were normal in appearance (Fig. 2). Bacteria were not found in the injured areas. This experiment was repeated at least 10 times, with similar results.

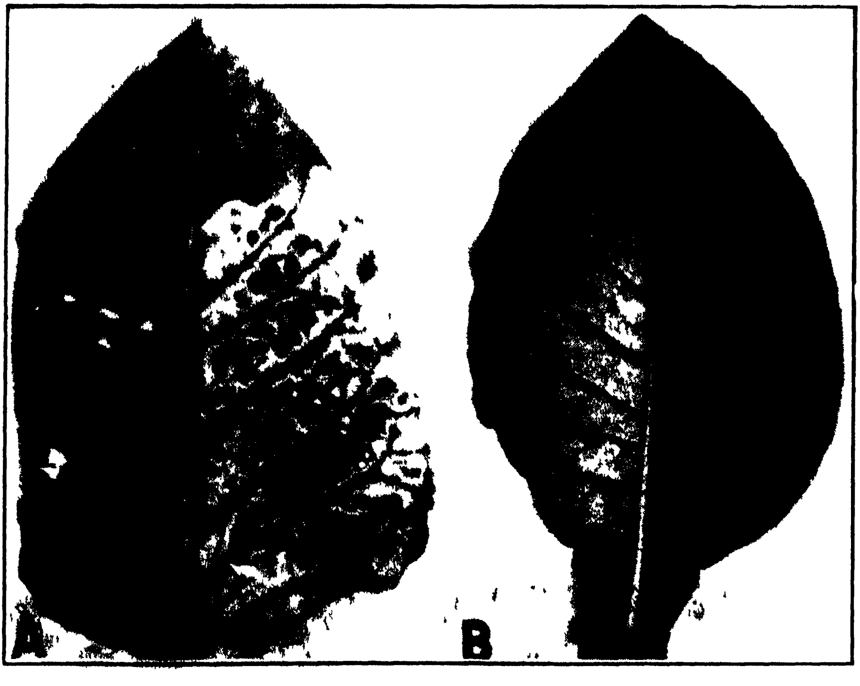

FIG. 3. A. The right side of the leaf was kept water-soaked 48 hours by keeping leaf submerged in pan of water after it had been water-soaked. Large chlorotic areas and necrotic spots resulted. Photographed 5 days after water-soaking. B. The left side of the leaf was water-soaked 72 hours; the leaf was kept attached to the plant; the root system was protected from water logging. The water-soaked area is chlorotic and nearly white.

Fig. 3, A, shows the chlorotic and necrotic brown areas on the water-soaked side of a leaf that was submerged in a pan of water 48 hours to maintain the water-soaked condition.

On June 6, 1938, one-half of each of 3 leaves on each of 4 potted plants was water-soaked with the nozzle 30 cm. from the lower surface. One plant was kept in the laboratory; the excess water was lost within 3 hours. The other 3 plants were placed in a moist chamber in which water was being sprayed; one plant was removed after 24 hours, a second after 48 hours, and the third after 72 hours. The excess water was lost two or three hours after removal from the chamber. Four days later the areas kept water-soaked 24 hours were chlorotic; those water-soaked 48 hours were chlorotic and necrotic; those water-soaked 72 hours were necrotic. All the areas water-soaked and allowed to lose the water at once were normal in appearance.

On June 16, 1938, the roots of 4 potted plants (40 cm. tall with about 14 leaves) were protected by covering the top of the pot with a paraffin paper seal. The plants were growing slowly and the bottom leaves were yellowing slightly. Half of each of 4 leaves of each plant was water-soaked in the usual manner. One plant was then left in the greenhouse atmosphere; the excess water was lost in about an hour. The other 3 plants were placed in mist formed by 4 spray nozzles. One plant was kept in the spray 24 hours, another 48 hours, the third 72 hours. Five days after water-soaking the areas kept water-soaked 24 hours or more were chlorotic, while the areas allowed to lose the excess water at once were normal in appearance. There was no necrosis on any of the leaves (Fig. 3, B). When this experiment was repeated on June 21, the results were similar.

Several times leaves of plants growing in the ground bench were water-soaked and kept water-soaked 48 hours by arranging spray nozzles to form a constant mist around the leaves. Mild chlorosis usually resulted, but severe necrosis did not develop. Apparently some factor such as temperature, maturity of the leaf, or physiological condition of the plant modifies the degree of injury. At times injury was severe and destructive; at other times very mild.

DISCUSSION

The results of these studies have demonstrated that water-soaking, continued for the 24 or 48 hours, claimed necessary to break down the resistance of tobacco leaves to the leaf spot organisms, caused extensive damage in itself without inoculation. In some instances the type of injury caused was identical in appearance with that caused by water-soaking and inoculation, as illustrated by both Clayton and J. Johnson. It cannot, therefore, be properly stated that water-soaking without inoculation does not cause injury. This is probably true only when the water-soaked leaves are allowed to give off the excess water soon after water-soaking has occurred.

If water-soaked tobacco tissue is inoculated with either *Bacterium tabacum* or *Bact. angulatum* and kept water-soaked for 24 hours or more there is no reason for believing that the combined effect of water-soaking and infection will not cause more extensive injury than water-soaking alone; but to claim that the mechanism by which outbreaks of wildfire and blackfire on topped tobacco are brought about has been discovered hardly follows. The type of injury pictured by both Clayton and J. Johnson as resulting from water-soaking and inoculation is not a type of injury commonly found in Kentucky on topped dark tobacco during outbreaks of blackfire. The typical blackfire spots, which are very nearly indistinguishable whether produced by *Bact. angulatum* or *Bact. tabacum,* are characterized by concentric zones of dead tissue. These zones are obviously not the result of rapid spread of bacteria in a water-soaked area in a period of two days, but require days and sometimes weeks to develop.

A further reason for not accepting the water-soaking hypothesis in connection with blackfire outbreaks is that it is extremely difficult to find water-soaked leaves even during such a season as that of 1938, when more than 40 per cent of the dark fired tobacco was lost from blackfire over an extended area in Western Kentucky. Continued search following storms failed to reveal more than an occasional water-soaked leaf during the summer but blackfire development was extensive.

CONCLUSIONS

It may be concluded that water-soaked tobacco leaves, if kept water-soaked for 24 to 48 hours, are likely to show extensive injury either at the end of the period or during the next few days, in the absence of infection with pathogenic bacteria. If the water-soaked tissues are inoculated, following water-soaking, injury will undoubtedly be increased but the injury thus produced bears no resemblance to the typical blackfire field injury as it occurs on maturing dark tobacco. It may, therefore, be stated that water-soaking plays little if any part in outbreaks of blackfire as they occur on maturing dark tobacco in Kentucky.

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WHITE STREAK, A VIRUS DISEASE OF NARCISSUS

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(Accepted for publication April 10, 1939)

White streak of narcissus was first observed in bulb plantings on Long Island in 1931, but in all probability it was present earlier. Owing to the fact that this disease has been confused with narcissus mosaic, very little information regarding it appears in the literature. Chittenden (2), reporting on studies made by W. E. H. Hodson, refers to the disease as "silver streak" and mentions its occurrence in Holland and England. McWhorter (5) reports the disease from the Pacific Northwest and calls it "white streak." Like the mosaic disease of narcissus, white streak is probably present wherever narcissi of commercial origin are grown.

SYMPTOMS

Chittenden (2) states that symptoms of white streak are manifest late in the season, whereas those of mosaic are prominent early. The writer, however, finds that the white-streak symptoms are also evident early in the season, but appear different from those expressed after the flowering period.

The early symptoms appear as narrow (0.5 to 2 mm. wide) dark green streaks of varying lengths (1 to 7 or more cm.), which are closely associated with the main vascular elements of the blade (Fig. 1, A and B). Later in the season these streaks become white, gray, or yellowish white and persist as such until the plant matures (Fig. 1, B and E). During the late stage of symptom expression two or more parallel streaks may coalesce (Fig. 1, E). The symptoms are very conspicuous also on the flower stems.

ETIOLOGY

Chittenden (2) and Caldwell and James (1) imply that the disease is probably caused by a virus. McWhorter (5) suggests that it "may be of virus origin or related to some elemental deficiency or to some asexual aberration accrued during bulb propagation."

The disease persists from year to year in the vegetatively propagated bulbs, a fact also reported by McWhorter (5); symptoms are of the mosaic type with which no visible organism has been associated; and the causal principle can be transmitted by mechanical methods. For these reasons the writer concludes that the disease is caused by a virus.

Mechanical Transmission. Owing to the fact that the narcissus mosaic virus can be easily transmitted mechanically the following methods were employed with the white-streak disease: Holmes' multiple insect pin method (3); the hypodermic needle method; and Rawlins and Tompkins' carborundum method (7)[1].

Healthy plants of the variety Minister Talma were grown in the greenhouse and indexed. Approximately, one per cent of the plants showed typical symptoms of the mosaic disease, whereas none showed symptoms of white streak. The healthy plants were inoculated with sap, diluted 1 to 5 with distilled water, which was extracted from leaves of King Alfred and Glory of Sassenheim plants showing late symptoms of white streak. Although the inoculated plants were observed at regular intervals until maturity, at no time did symptoms develop. The dormant bulbs were left, therefore, in the original flats and regrown a year later. At this time definite, early symptoms of white streak were apparent in several of the plants (Table 1).[2] The carborundum method of inoculation proved superior to the hypodermic needle method and both were much better than the multiple insect pin method.

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[1] Haasis, F. A. Studies on narcissus mosaic. Cornell University Agr. Exp. Stat. Mem. 224.

[2] White streak symptoms, in the variety Minister Talma, have been observed only on experimentally infected plants. The early symptoms are identical with those on naturally infected King Alfred, Victoria, and Glory of Sassenheim plants; the late symptoms, however, are greenish yellow in the variety Minister Talma rather than white, gray or yellowish white.

TABLE 1.—*Transmission of the white streak virus of Narcissus*

Source of inoculum	Methods of inoculation	Transmissions over number of healthy Minister Talma plants inoculated			
		Test number			
		1	2	3	4
Diseased King Alfred	Holmes', 100 to 150 punctures in 3–4 leaves	0/19	0/19		
Diseased Glory of Sassenheim	"	0/20	1/20		
Diseased King Alfred	Hypo. needle, 1/3 cc. to 3–4 leaves	3/20	3/19		
Diseased Glory of Sassenheim	"	4/18	2/20		
Diseased King Alfred	Rawlins and Tompkins' 3–5 leaves rubbed	4/19	6/19	5/18	4/20
Diseased Glory of Sassenheim	"	8/18	6/18	8/18	6/16
Distilled water	Holmes', same as above	0/17			
"	Hypo. needle, same as above	1/19[a]			
"	Rawlins and Tompkins', same as above	0/18			
Uninoculated	None	0/20			

[a] Transmission by cutting knife suggested here; the flowers of these plants were cut by greenhouse attendants.

EPIPHYTOLOGY

Since the late stage of symptom expression does not develop, under field conditions, until 3 to 4 or more weeks after blooming, it was thought that temperature might play a part in symptom development. In order to determine this point, Bicolor Victoria plants showing late symptoms of the white-streak disease were rogued from the field and the bulbs later planted in porous-clay pots. The plants were forced in greenhouses maintained at the following minimum temperatures: 40, 50, 58, 68, and 80 degrees F. The late type of symptom expression showed only in the plants placed in houses with minimum temperatures of 68 and 80 degrees F. It, therefore, appears that the late symptoms of the disease are conditioned by temperatures in excess of approximately 65° F.

CONTROL

The only known method of controlling this disease at the present time is that of roguing (5). However, owing to the fact that the disease is present in high proportions in many narcissus bulb stocks, the inverse method of roguing, suggested by McWhorter and Weiss (6) for mosaic control, under

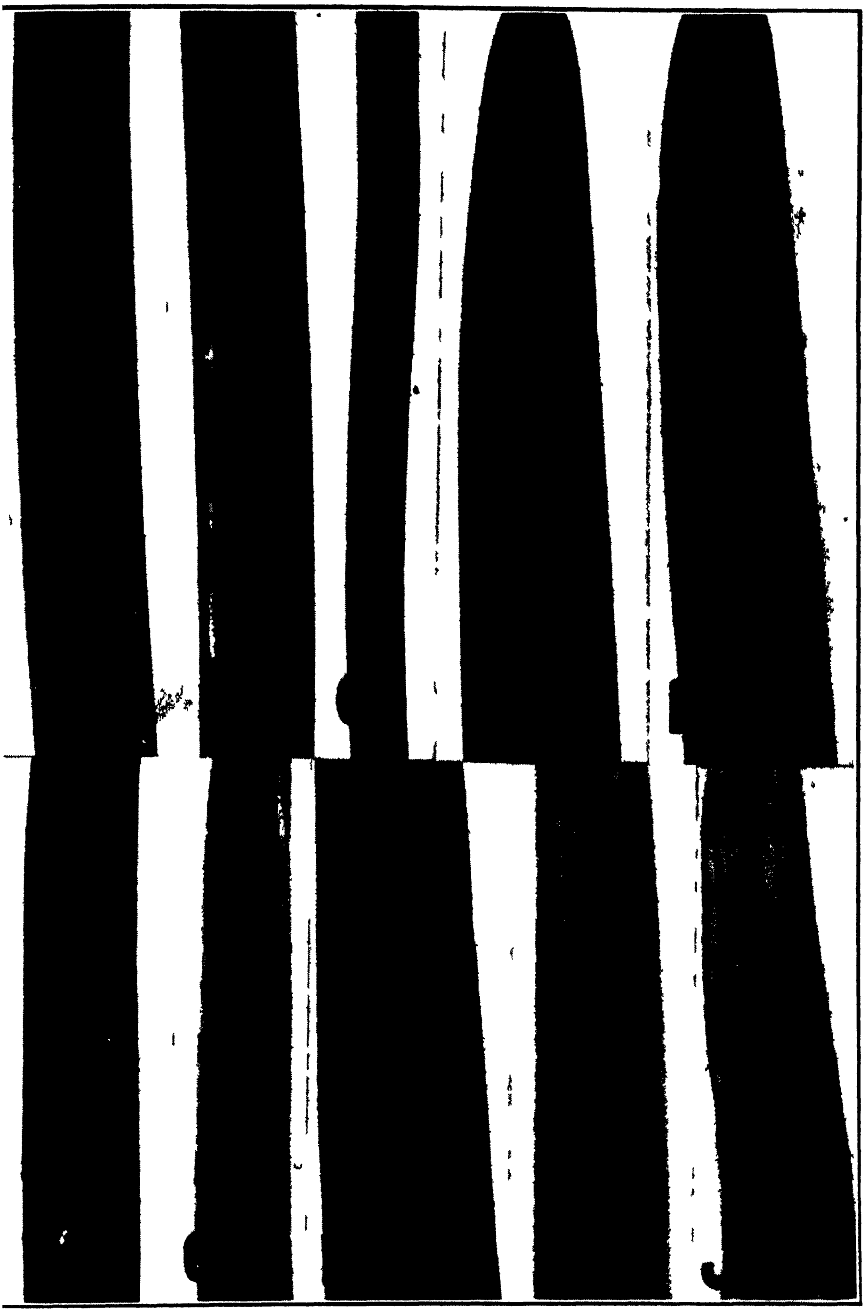

FIG 1 White streak and mosaic symptoms on narcissus foliage. A. King Alfred leaf showing early symptoms of white streak—naturally infected. B. Same as A except late symptoms beginning to develop. C. Late symptoms on leaf of Minister Talma—experimentally infected D Healthy Victoria leaf. E. Late symptoms of white streak on Victoria, naturally infected F. Healthy King Alfred. G. Mosaic on King Alfred. H. Healthy Sir Watkin I. Mosaic on Sir Watkin. J. Mosaic on Victoria.

certain conditions, may prove more economical and equally as effective. One interested in ridding his plantings of white streak plants should learn to diagnose the early symptoms of the disease, for by so doing he will be enabled to rogue before the natural agents of inoculation have much opportunity to work. Furthermore, foundation plantings of healthy stock should be far removed from all other narcissi, in order thus to escape, or at least minimize, reinfections.

DISCUSSION

White streak differs from mosaic in that mosaic symptoms are evident as light green to greenish yellow stripes and blotches (Fig. 1, G, I, J) from the time of foliage emergence until late in the season, whereas the symptoms of white streak, although present early in the season as narrow dark green streaks, do not become prominent until 3 to 4 or more weeks following the blooming period, when the symptoms appear as white, gray, or yellowish white streaks (Fig. 1, A, B, E); the foliage of mosaic-diseased plants usually is roughened as a result of cellular outgrowths, but this is not the case with the white-streak disease; and high temperatures have little effect on mosaic-symptom expression whereas high temperatures intensify the symptoms of the white-streak disease. It is believed, therefore, that the virus causing white streak differs from that causing the mosaic disease of narcissi.

Whether or not the two diseases may be due to virus strains, which are closely related, is a matter of speculation; observational evidence, however, tends to support such a theory. Individual narcissus plants showing symptoms of both white streak and mosaic have been only occasionally encountered. Thus it may be that a plant once invaded by one of the viruses, is rendered immune from subsequent infection with the other; Kunkel (4) has reported such a relationship between the viruses causing peach yellows and little peach. The fact that an occasional narcissus plant shows symptoms of both diseases may be explained on the basis of simultaneous inoculation with the two viruses, or successive inoculations separated by short intervals. Salaman (8) has demonstrated that plants inoculated successively, with closely related virus strains, but the successive inoculations separated by short intervals (from 1 to 8 days), do not show the immunity reaction, whereas plants receiving successive inoculations, separated by longer intervals (in excess of 8 days), develop immunity from the strain used for the second inoculation.

SUMMARY

White streak is an infectious disease of narcissi caused by a virus. The virus is mechanically transmissible.

The white-streak virus is considered to be different from the mosaic virus of narcissi, the difference being based on symptom expression. Observational evidence supports the theory that the two viruses may be closely related.

The only known method of control at the present time is eradication of diseased plants and isolation of healthy plants.

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RENEWED LIQUID-CULTURES OF FUNGI

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(Accepted for publication April 10, 1939)

The problem of physiological studies in the growth of fungi has been given attention by many investigators, but every one has realized the limitations of a solid medium or of unchanged liquid-cultures in such work. Liquid cultures naturally offer a solution to the problem if they can be constantly renewed and yet remain sterile, except for the initial inoculum. The apparatus illustrated in figure 1 yields a solution to this intriguing problem, and data are submitted to substantiate the very successful operation.

The basic principle of the apparatus is not new, having been described by Wean,[1] but modifications have been made in adapting the flow-meter to control the flow of nutrients into fungus cultures. Since the apparatus must not become contaminated during operation, all corks were of rubber. Where air was allowed to enter the system a cotton filter was provided, *i.e.*, at *E* of the flow-meter and *J* of the culture flasks.

The nutrient chamber *B* is a six-liter Pyrex flask, connected to flow-meter *F* by a siphon tube *D*. The intermediate chamber *F* insures a constant "head" and uniform rate of flow of the nutrient, for the air tube *C* regulates the operation of the siphon. Although all nutrients were filtered, a sediment flask *G* was installed to eliminate the chance of sediment-clog-

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[1] Wean, Robert E. Automatic flow-meter for drip solutions in plant nutritional studies. Science 82: 336. 1935.

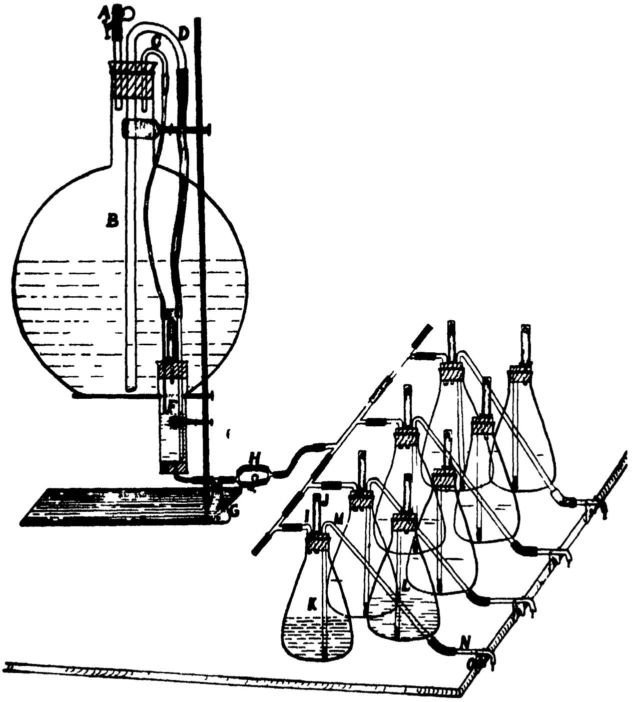

FIG. 1. Apparatus for the automatic control of the flow of nutrients into fungus cultures.

ging the system at any point. The double unit *H* provides on the clamp-side a direct passage for nutrient to start the drip through the capillary tube *I* into the flasks *K* and for flushing at intervals. When the clamp is in position the solution passes through a 0.50 mm.-bore glass tube on the opposite side, which reduces the rate of flow to the flasks.

The fresh solution drips into the flasks of 500 cc. volume on the upper surface of the medium, while the portion removed is taken from the bottom by a siphon tube *M*. The discharge from tube *M* is controlled by the 0.50 mm.-bore tube *N* the tip of which may be raised or lowered by the sliding block *O*. A filter of glass wool in the tube *M* prevents the fungus hyphae from stopping the discharge.

During the experiment, 400 cc. of nutrient passed through each renewed culture every 24 hours. The control flasks *L* were identical to those of *K*, except for the renewal of the solution. The initial amount of nutrient in each was 150 cc.

Prior to autoclaving the apparatus, the system was started as for operation with the nutrient levels as desired and reservoir *B* nearly full. In autoclaving, the 4 flasks were separated from the system at the glass *T* above point *H* and the glass was capped by a cotton filter. The rubber connection was plugged by a solid glass rod. Care must be exercised that the tip of air tube *C* is not submerged and that siphon tube *D* is shut off by a clamp. After autoclaving, it is a simple matter to connect the system and remove the clamp from tube *D*, which places the system in operation. In the event additional nutrients need to be introduced into the reservoir, tube *D* is closed by a clamp, tube *C* is allowed to become uncovered in chamber *F*, and the plug and clamp of tube *A* are removed.

The data herein reported were secured by growing *Pythium debaryanum* in the liquid cultures for a period of 10 days. The fungus was grown on prune agar, and hyphal tips of the advancing mycelium were used in a block 6 mm. sq. for inoculating each flask. After inoculation, the flasks were allowed to stand 4 days before beginning the renewal of the nutrient. They were shaded from direct light at all times. Supplementary tests have shown that this organism gives a regular incremental increase in growth on Richard's solution throughout the initial 4-day period.

Richard's solution, as used at full concentration, consisted of the following: 10 g. KNO_3, 5 g. KH_2PO_4, 2.5 g. $MgSO_4$, 0.02 g. $FeCl_2$, 50 g. $C_{12}H_{22}O_{11}$ and 1000 cc. distilled water. The pH of the solution, as made up and autoclaved, was 4.7. The concentrations of this solution used in the experiment were full, $\frac{1}{2}$, $\frac{1}{8}$, and $\frac{1}{32}$. The data presented are based upon 8 flasks for each of the above dilutions—4 controls and 4 with the nutrient renewed. More flasks could easily be added to the system without disturbing its operation.

The growth of the fungus was determined at the end of a 10-day period

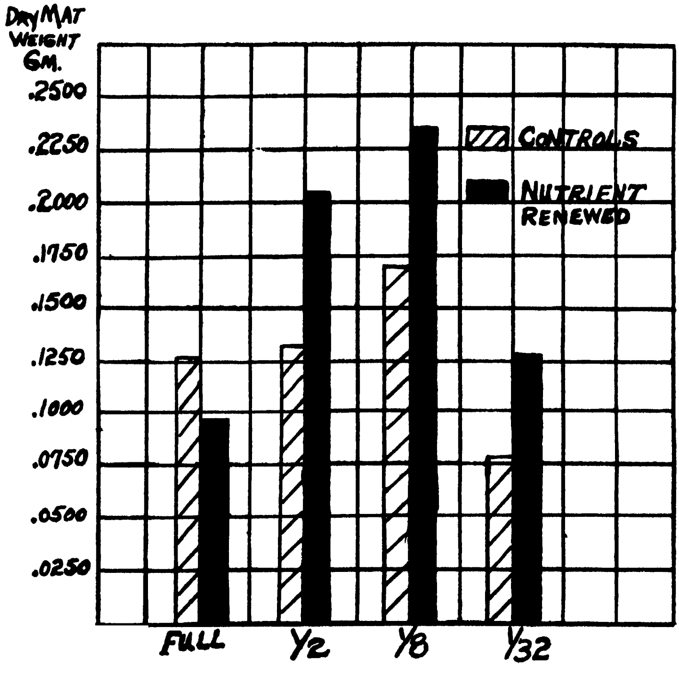

FIG. 2. Graphic presentation of the effects on fungal growth resulting from renewal of nutrient at different concentrations.

of growth by placing the mats in previously weighed Gooch crucibles, removing all possible moisture by a suction pump, and drying 8 days at 48° C.

In figure 2 the effects of renewing the nutrient at the various concentrations are illustrated. By maintaining the full nutrient at pH 4.7, growth was less in the renewed solutions than in the controls. Concentration and availability, no doubt, explain the reaction. The increased growth secured by renewing the solutions of $\frac{1}{2}$ and $\frac{1}{8}$ concentrations was nearly the same, while at $\frac{1}{32}$, the increased growth was easily visible, although the concentration was too low for maximum growth. These results plainly demonstrate the value of such an apparatus for studies of fungus growth and that only by such technique can data of accurate nature be ascertained.

Through using a renewal rate of 400 cc. per 24-hour period it was possible to maintain the pH of the solutions, except at the greatest dilution, where a drift to 5.1 was recorded. Evidently the rate of renewal needs to be increased at such great dilutions. This drift, however, need not affect the significance of the data when the pH of the controls is studied. As a result of fungus activity the drift over a 10-day period was as follows: full nutrient to pH 5.50, one-half conc. 5.84, one-eighth conc. 6.28, and one thirty-second conc. to 6.72. Without doubt these changes in acidity reflect the extensive alterations occurring in an unchanged medium as a result of metabolic processes.

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CHOANEPHORA CUCURBITARUM ATTACKING COWPEAS

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(Accepted for publication April 27, 1939)

In 1917 Wolf[2] reported *Choanephora cucurbitarum* (Berk. and Rav.) Thax. on fading flowers of the cucumber, althaea, scarlet hibiscus, okra, cotton, and squash, but considered it parasitic only on squash. Wolf and Lehman[3] mention finding this fungus on cowpeas, *Vigna sinensis* (Torner) Savi, in North Carolina. In September 1937, at Experiment, Georgia, the writers found a fungus causing a decay of the pods of Groit cowpeas, which was tentatively identified as *C. cucurbitarum*. About 5 per cent of the pods in this field were affected. The part of the pod attacked had a water-soaked appearance, and the fungus fruited abundantly on the surface (Fig. 1). The infected tissues were somewhat softened and the pods were often constricted, due either to the retardation of growth or to the drying out of the affected parts.

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[1] The writers are indebted to Drs. D. H. Linder and T. T. Ayers for furnishing cultures of *Choanephora cucurbitarum* for comparative study.

[2] Wolf, F. A. A squash disease caused by *Choanephora cucurbitarum*. Jour. Agr. Res. [U.S.] 8: 319–328. 1917.

[3] Wolf, F. A. and S. G. Lehman. Notes on new or little known plant diseases in North Carolina in 1920. Ann. Rept. N. C. Agr. Exp. Stat. 43: 55–58. 1920.

FIG. 1. Infected pods of Groit cowpea showing the grayish-white mycelium and the darker conidal heads of *Choanephora cucurbitarum.* × ⅞.

Again in the summer of 1938, *Choanephora cucurbitarum* was found on cowpeas at the same station, but it caused very little damage because of the unusually dry season. Even under such adverse conditions, the following varieties were found to have one or more infected pods to the rod row: Groit, 3 pods; Brown Sugar Crowder, 2; Etheridge, 2; Crop Crowder, 4; Jumbo Blackeye, 1; Conch, 1: Virginia Blackeye, 1; Red Hulled Speckled, 1; and California Blackeye, 1. Observations made by the pickers at the time of picking indicated that the variety Brown Sugar Crowder was one of the most susceptible. They also noticed that if the cowpeas were picked a little too green and stored in a tight bag, the disease developed rapidly and caused considerable damage. Because of this type of injury, the disease may be considered a storage rot and might cause damage in transit to cowpeas picked and shipped in the pods. The disease has not been observed, however, on cowpeas in the market.

Field observations indicated that the fungus apparently attacked partly ripened pods most frequently, and rarely attacked entirely green, growing tissue. Mature pods touching the ground were commonly attacked, and pods a foot or so above the ground occasionally were infected. The fungus was very active under a dense foliage during damp, foggy weather. Blossoms that had fallen off and were lying on the leaves of the plant or on the soil were commonly infected.

Several inoculation experiments were conducted to determine the pathogenicity of the fungus. In the first experiment, pods of different ages, from half-grown to nearly mature, were placed in two moist chambers. Those in one were inoculated by inserting a small amount of an agar culture of the fungus into a small slit in each pod and those in the other were wounded similarly but not inoculated. It was found that only the nearly mature inoculated pods became infected and decayed, while the younger green pods, as well as those not inoculated, were not attacked.

In another experiment, 5 Blackeye cowpea pods, still attached to the plant, were inoculated and incubated as described above, with the fungus isolated from cowpeas from Georgia, while 5 pods on another plant of the same variety were inoculated with *Choanephora cucurbitarum* isolated from summer squash by T. T Ayers. After 5 days, 3 of the more mature pods, although still green, showed water-soaked lesions, and the fungus was producing conidial heads abundantly on the affected areas. The other 2 pods, as well as the 5 inoculated with the *C. cucurbitarum* from squash, remained free from infection, even after remaining in the incubation chamber 12 days. The control plants also showed no infection. From the above field observations and laboratory experiments it is evident that the fungus is a weak parasite on the pods of cowpeas

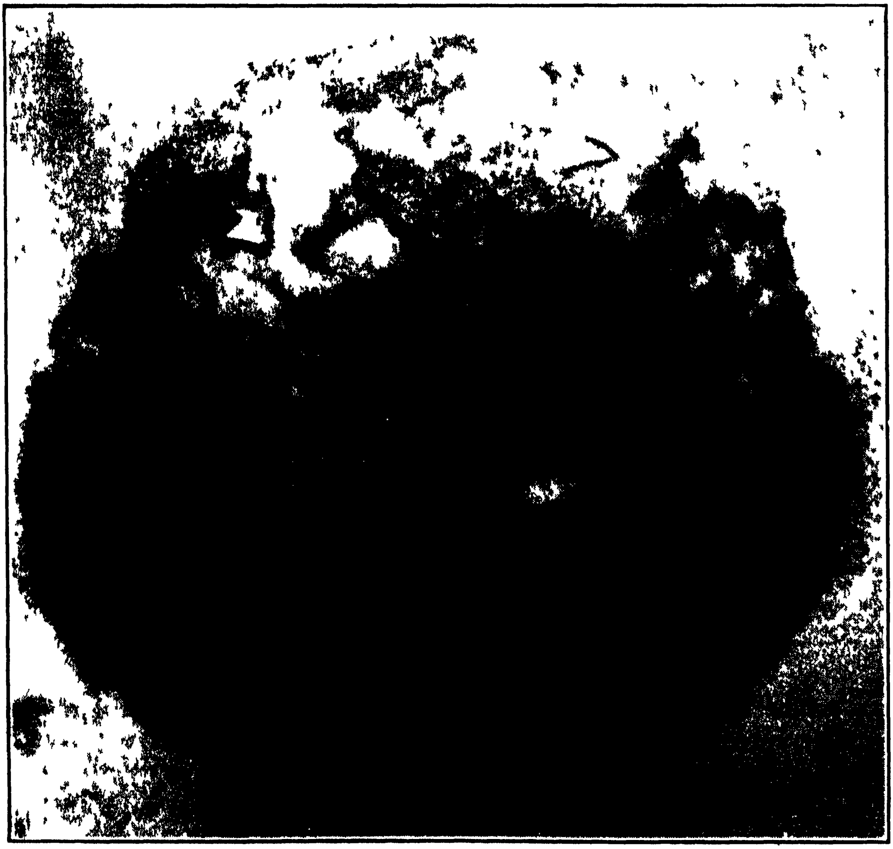

FIG. 2. Cymling squash, artificially inoculated with *Choanephora cucurbitarum*, showing the grayish-white mycelium and the dark conidal heads of the fungus. ×1.

In still another experiment, 9 acorn squashes, 3 cymling squashes, and 2 summer squashes were inoculated with the *Choanephora cucurbitarum* isolated from cowpeas. The squashes were inoculated by cutting out small plugs from each squash and inserting a small portion of the pure agar culture of the fungus into each hole. The plugs were then replaced and the squashes were placed in a moist chamber. Adequate controls were maintained. After 3 days the fungus was fruiting abundantly upon the water-soaked and softened tissue of the inoculated cymling (Fig. 2) and summer squashes. All the Acorn squashes remained free from infection. even though kept in the moist chamber 10 days. The controls of the different varieties also remained free from infection.

To verify the identification of the fungus isolated from cowpeas, cultures of *Choanephora cucurbitarum* were obtained from D. H. Linder and T. T. Ayers for comparative study. The fruiting structures from these various cultures were found to be very similar; their measurements agreed fairly well with those given by Wolf.[4] The sporangia of the fungus from cowpeas ranged from 40 to 80 μ; the sporangiospores, 17–37 × 10–16 μ, averaging 26 × 13.5 μ; and the conidia, 16–37 × 8–16 μ, averaging 20.8 × 12 μ. It was found, however, that when the sporangiospores from the 3 sources mentioned above were examined under the oil-immersion objective, the spore walls showed very fine longitudinal striations. As these markings on the spore walls have not been reported previously, attention is called to their presence.

In addition to finding *Choanephora cucurbitarum* on cowpeas in Georgia, the fungus also has been observed commonly as a saprophyte on leaves of various grasses collected in Georgia and Florida, when such grass leaves have been incubated in moist chambers.

COOPERATIVE INVESTIGATIONS BETWEEN THE DIVISION OF
FORAGE CROPS AND DISEASES, BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE, AND THE GEORGIA
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ACCELERATION OF TOXIMETRIC TESTS OF WOOD PRESERVATIVES BY THE USE OF SOIL AS A MEDIUM

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(Accepted for publication April 17, 1939)

Malt agar, containing different concentrations of the preservative under test, has been used, in the United States, as the standard for the assay of wood preservatives.[1] In Europe use of impregnated wood blocks, laid upon the surface of vigorously growing fungi in kolle flasks,[2] has been the preferred technique. This latter method has the advantage of using the material to be preserved as part of the substrate. The equipment, however, is expensive and fragile, the technique somewhat cumbersome, and the results are usually not very consistent. In an effort to increase the efficacy of this type of test a modification requiring less expensive apparatus and simpler techniques has been evolved.[3] Unfortunately, this method still leaves much to be desired in regard to uniformity of result and vigor of attack, especially by organisms of the *Hausschwamm* type.

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[4] See footnote 1, page 898.

[1] Fleming, Ruth M., and C. J. Humphrey. Toxicity of various wood preservatives II. Jour. Ind. and Eng. Chem. 7: 652–658. 1915. Schmitz, Henry, *et al.* A suggested toximetric method for wood preservatives. Ind. Eng. Chem. (Anal. Ed.) 2: 361–363. 1930.

While conducting some experiments with subterranean termites, sterilized blocks of southern yellow pine (*Pinus echinata*) sapwood, placed in a large tank and covered with rich garden soil, were infested with local termites (*Reticulitermes flavipes*) by placing small pieces of infested wood on the soil. Two months later, following a routine examination of the termite colonies, it was noticed that, while some of the wood had been attacked by termites, every specimen showed vigorous fungous attack. This was by far the most rapid and consistent decay that had been effected in these laboratories.

The possibility that any especially virulent organism was responsible was first investigated. Untreated blocks of southern yellow pine sapwood (*Pinus echinata*—2 × 2 × 2 cm.) were placed in small wide-mouth screw-cap bottles, covered with soil, and the entire set-up sterilized. The blocks were then inoculated with pure cultures of our most common test organisms. The fact that the decay was again unusually rapid and uniform indicated that the test conditions rather than the organism were the decisive factor. The results on untreated blocks with the fungi *Fomes roseus, Polyporus vaporarius* and *Poria incrassata,* as compared with similar blocks and the same organisms using the former technique,[4] given in the accompanying table, are

TABLE 1.—*Comparison of weight losses obtained in untreated blocks by revised (soil) and former assay method*

Time in test weeks	*Fomes roseus*					*Polyporus vaporarius*					*Poria incrassata*				
	Weight loss in per cent					Weight loss in per cent					Weight loss in per cent				
	New method			Old method		New method			Old method		New method			Old method	
4	6.6[a]	8.3	9.1	Nil	Nil	Nil	Nil	Nil	Nil	Nil	1.0	6.4	10.4	Nil	Nil
8	20.6	21.3	22.1	4.7	7.5	17.2	19.6	26.8	4.2	19.7	28.1	30.5	33.8	Nil	Nil
12	26.1	31.8	34.1	3.8	4.7	27.9	35.3	39.2	9.1	14.6	38.0	45.1	47.1	7.5	10.4
16	30.0	32.2	34.7	11.0	11.8	37.8	41.2	41.8	10.9	12.7	47.5	47.6	47.8	13.9	14.4
24				28.3	29.4				18.3	20.4	. ..	.		13.5	17.8
48														20.7	23.5

[a] Each result represents an individual block.

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[2] Findlay, W. P. K. Laboratory methods for testing wood preservatives. Ann. Appl. Biol. 19: 271–280. 1932. Liese, J., *et al.* Toximetrische Bestimmung von Holzkonservierungsmitteln. Z. Angew. Chem. 48: 21–23. 1935.

[3] Waterman, R. E., John Leutritz, and Caleb M. Hill. Chemical studies of wood preservation. The wood block method of assay. Ind. and Eng. Chem. 10: 306–314. 1938.

[4] Waterman, R. E., John Leutritz, and Caleb M. Hill. A laboratory evaluation of wood preservatives. Bell Syst. Tech. Jour. 25: 194–211. 1937.

of interest not only because they illustrate the high degree of uniformity and rapidity of attack obtainable by this technique but also because they represent by far the maximum decay obtained on untreated controls in these laboratories using organisms of the *Hausschwamm* type.

Previous experience has taught that moisture control is of great importance in wood-rotting tests. The moisture content of the soil used was 20 per cent on a dry-weight basis, just about enough to cause the soil to cohere when squeezed in the hand. Noninoculated blocks in contact with this soil uniformly come to a moisture equilibrium slightly above fiber saturation; decayed blocks become much wetter on a dry-weight basis, but the *total* water present in the block is not much higher than in the noninoculated controls. Many methods have been tried during the past few years to bring about uniform moisture control at the point optimum for decay, but this simple expedient gives unquestionably the best results.

This improved moisture control may possibly be the sole cause of the consistently accelerated decay. The possibility still remains, however, that at least part of the beneficial result is due to nutrilites, organic or inorganic, present in the soil. The fact that the greatest weight losses encountered, using the former bottle technique with the *Hausschwamm* type of organism, was on blocks treated with low concentrations of some salt-type preservatives would indicate that a major effect of the soil is the introduction of mineral constituents. Further studies are being made to elucidate the phenomena involved and to develop a new and better standardized assay method for wood preservatives.

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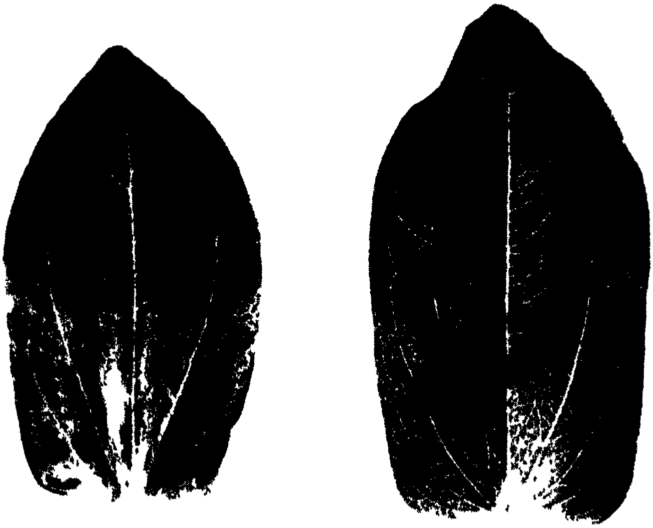
PHYTOPATHOLOGICAL NOTES

Cross Protection Tests with Two Strains of Cucumber-Mosaic Virus.—Cucumber mosaic was first described in 1903 by Selby (8) and later shown to be an infectious disease by Doolittle (2) and Jagger (3), who, independently, demonstrated it to be caused by a filterable virus. Strains of the virus are now known to occur in many different kinds of plants. In the writer's studies on differentiation and classification of cucumber-mosaic viruses (5, 7), the "white pickle" virus of Porter (4) was used as the type because of its availability at the time. Moreover, its symptomatology, host range, and properties led to the assumption that it was closely related to the strain studied by Doolittle. The assumed relationship, however, has been called into question by Chester's (1) failure to obtain a serological reaction between the two viruses and, to a lesser extent, by the fact that Doolittle's virus produces a more severe disease in tobacco (*Nicotiana tabacum* L.), tomato (*Lycopersicon esculentum* Mill.), zinnia (*Zinnia elegans* Jacq.), and *Nicotiana glutinosa* L. It is important to know if there are 2 cucumber-mosaic-virus groups existing in the United States. Experiments were, therefore, undertaken to determine whether or not Porter's and Doolittle's cucumber-mosaic viruses should be classified in different virus groups. This paper presents the results of the experiments.

It has been shown (6) that zinnia plants, mottled by any one of a number of different strains of Porter's cucumber-mosaic virus, are protected from strain 6 of this virus, a strain that produces necrotic primary lesions in healthy zinnias and in those mottled by unrelated viruses. Cross protection tests in zinnia thus afford a convenient method for identifying viruses of the cucumber-mosaic group. In the present work, Doolittle's cucumber-mosaic virus was transferred to young Golden Gem zinnia plants. It produced a systemic mottling disease in these plants and caused marginal necrosis in some leaves. When the plants had become thoroughly mottled they were dusted with carborundum powder and from 4 to 6 leaves on each plant were rubbed with strain 6 virus. At the same time, an equal number of similar leaves of healthy zinnia plants were rubbed in the same manner. After 8 days, the necrotic lesions that had developed were counted and tabulated. In one experiment a total of 413 necrotic lesions developed on 22 previously healthy leaves, but not a single lesion appeared on 22 mottled leaves (Fig. 1).



Photograph by J. A. Carlile

FIG. 1. Zinnia leaves 8 days after inoculation with No. 6 strain of cucumber-mosaic virus. Necrotic lesions were produced on the previously healthy leaf (right) but not on the leaf mottled by Doolittle's cucumber-mosaic virus (left).

In a second experiment 1,324 lesions developed on 56 control leaves and no lesions on an equal number of mottled leaves. The data show that zinnia leaves, mottled by Doolittle's virus, are solidly immune from strain 6 virus and thus bring evidence that the two viruses are closely related. It is concluded that, in spite of their symptomatological and serological differences, Porter's and Doolittle's cucumber-mosaic viruses should be classified in the same virus group.—W. C. PRICE, Department of Animal and Plant Pathology, The Rockefeller Institute for Medical Research, Princeton, New Jersey.

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5. PRICE, W. C. Isolation and study of some yellow strains of cucumber mosaic. Phytopath. 24: 743–761. 1934.
6. ———. Acquired immunity from cucumber mosaic in zinnia. Phytopath. 25: 776–789. 1935.
7. ———. Classification of lily mosaic virus. Phytopath. 27: 561–569. 1937.
8. SELBY, A. D. Report of committee on vegetable pathology. A peculiar malady of forced cucumbers. Ann. Rept. Ohio State Hort. Soc. 1902: 109. 1903.

Preliminary Observations on a Kernel Discoloration in Inbred and Hybrid Lines of Dent Corn.[1]—An apparently unusual discoloration of the crowns of corn kernels was called to the writer's attention on a number of occasions during the harvest season of 1938. This condition was observed in the nursery at Lafayette, Indiana, and in Illinois on inbred 66; and on single crosses involving inbred Hy, *i.e.*, 38–11 × Hy and L317 × Hy.[2] At Kentland, Indiana, the discoloration was prevalent on hybrid L317 ×ITE701.

The discoloration varied from a light tan to a medium brown and was confined primarily to the crown of the kernel as shown in figure 1A. Occasionally, the discolored area extended a short distance down the faces of the kernel. In one unidentified white corn, illustrated in figure 1A, the pattern of discoloration was a well-defined brown streak running over the center of the crown and parallel to the long axis of the ear.

Since the condition has been reported to detract markedly from the sale value of seed corn, investigations were undertaken to determine its nature. Normal kernels and those showing all gradations of discoloration were removed from the ears, surface-sterilized in a sodium hypochlorite solution, and germinated on agar plates and in rag-dolls. From these tests there was no indication that the vitality of the discolored kernels had been impaired; germination was as vigorous in these as in kernels of normal color. Platings were made of surface-sterilized kernels on potato-dextrose agar and on beef-peptone agar adjusted to different pH levels. Following surface sterilization an incision was made in the crowns of some of the kernels

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[1] Cooperative investigations, Purdue University Agricultural Experiment Station and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

[2] The writer is indebted to Mr. R. O. Snelling and Mr. R. R. St. John for supplying some of the material used in this study.

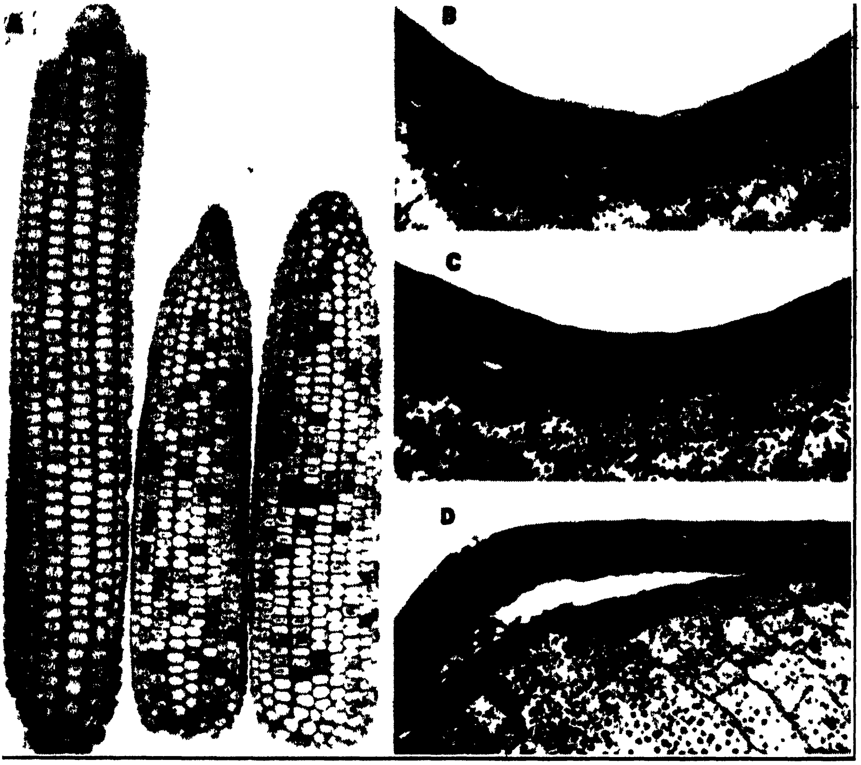

FIG. 1. A. Symptoms of kernel discoloration of corn. The ear on the left is an unidentified white corn. The other 2 ears are of the single cross, 38–11 x Hy. B. Longitudinal section through crown of a normal kernel showing well-developed aleurone layer. C. Longitudinal section through crown of discolored kernel showing the absence of the aleurone. D. Section through a corner of the crown of a discolored kernel showing absence of aleurone at the right in the discolored area.

with a flamed scalpel. The results of the platings suggested that the condition could not be attributed to fungi or bacteria propagated by ordinary methods.

Further observations were made on serial sections of fixed and imbedded kernels. A comparative study of sections of normal (Fig. 1B) and discolored kernels (Fig. 1C and D) showed the aleurone layer to be absent in those areas where the brown coloration was in evidence. The starch-bearing endosperm cells abutted directly onto the pericarp and took on a deep stain in safranin. Whether the formation of the aleurone is prevented in the early stages of development of the kernel or is destroyed by enzymatic action is unknown. Observations thus far suggest some type of enzymatic breakdown as responsible for the absence of the layer. The same histological picture was found in the white corn, as might be expected, except that the area in which the aleurone was absent was considerably smaller. Further studies are planned for the purpose of observing the progressive development of the condition, and the possible factors contributing to its manifestation.—**ARNOLD J. ULLSTRUP**,[3] Purdue University Agricultural Experiment Station, Lafayette, Indiana.

Lesions on Quercus laurifolia Similar to Those of Leprosis on Citrus in Florida.—In 1932, while on a trip through the citrus sections of Florida, lesions closely resembling those of leprosis on citrus were observed on young trees of laurel oak (*Quercus laurifolia* Michx.)[1] occurring on small branches and twigs (Fig. 1). The intention was to publish this note in 1936,[2] but the publication of it has been delayed.

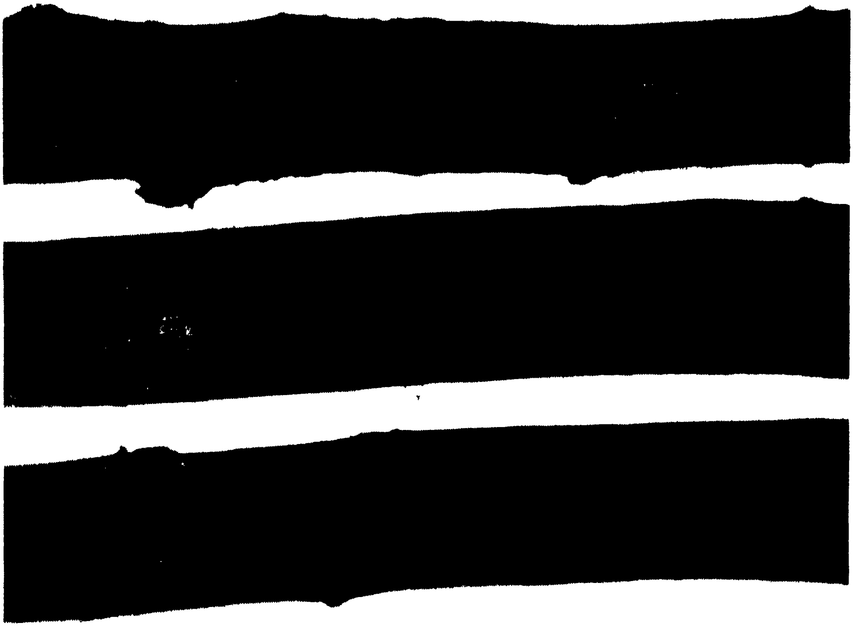

FIG. 1. Lesions resembling leprosis on *Quercus laurifolia*, growing near a neglected sweet-orange tree, severely attacked by leprosis. Indianola, Merritt Island, Florida. Natural size. Photo by A. S. Rhoads.

These lesions were first found on January 22 in an old, neglected property on the east bank of the Indian River at Indianola, Merritt Island, on the east coast of Florida. They occurred in close proximity to orange trees on which leprosis had flourished for several years, the property having grown up in a thicket of hammock-type forest. Similar lesions were found subsequently by the senior writer on the same species of oak at other points in Florida, mostly in proximity to leprosis on citrus, in the vicinities of Bay-view, Dunedin, and Sutherland, Pinellas County, on the west coast, and also at Valrico, Hillsborough County, and Wildwood, Sumter County. However, they were not found on this oak at Gainesville, where leprosis on citrus is not known to occur.

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[3] Agent, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture.

[1] Distribution of this species, according to A. W. Chapman, is from the Carolinas to Biscayne Bay, Coloosbatchie River to Louisiana, and thence inland for some distance.

[2] Fawcett, H. S. Citrus diseases and their control. McGraw-Hill Book Co. (New York) 656 pp. (*See* p. 257 and footnote.) 1936.

These lesions on the oak were strikingly similar to those of leprosis on nearby citrus trees. It, therefore, seems possible that they may be due to similar causes. From the general character of leprosis on citrus[3] it is suspected that it may also be a virosis.[4,5] Whether or not the lesions on the oak and citrus are attributable to the same cause remains to be determined. It is of interest to note that nearly all of the cases of leprosis in Florida are in fairly close proximity to bodies of water and that all of these oaks on which lesions were found were located also in similar situations. This may be merely a coincidence or might suggest that if an agent of transmission is found later, it will be one favored by moist situations near bodies of water.

It may be pointed out that leprosis in Brazil, although found often in severe form near bodies of water, is found also in other situations, as well. In northern Argentina and Paraguay, leprosis (lepra explosiva) is abundant on citrus, often near bodies of water but not exclusively in such situations.—H. S. FAWCETT AND A. S. RHOADS, University of California Citrus Experiment Station and Florida Agricultural Experiment Station Citrus Field Laboratory, Riverside, California, and Cocoa, Florida.

An Improved Transfer Hood.—Many phytopathologists, mycologists, and bacteriologists employ a small hood as an aid in the avoidance of contamination during isolation and transfer of pure cultures. Such a hood is inexpensive, economical of space, and portable (enabling its use in temporary field headquarters). The writer has recently designed a transfer hood incorporating certain improvements (see figure). The glass top slopes toward the operator, providing a convenient view of the interior and space for burner-heated air in the gable, whence it escapes through holes in the sides; electric lights at the base of the top provide illumination; and an enameled table top replaces a fixed bottom.

The transfer hood (Fig 1), finished with an acid-resistant white paint, is 20″ × 36″ beneath, rising 12″ in front and 30″ behind, the top thus sloping at an angle of 48 degrees. The rear and top are of glass; each side is made from a single piece of five-ply wood equipped with a 10″ × 10″ glass door; and the front is sealed by a taut piece of closely woven, stout cloth ("silkaleen"), with 12″ sleeves measuring 6″ × 10″ in diameter at the elliptical proximal end and 6″ at the circular distal end, which is elastic to fit snugly to the elbow. The blood is bottomless and rests on an enameled-top, kitchen table, close contact being assured by a pneumatic rubber, refrigerator-door gasket glued to the bottom edges (*a*).

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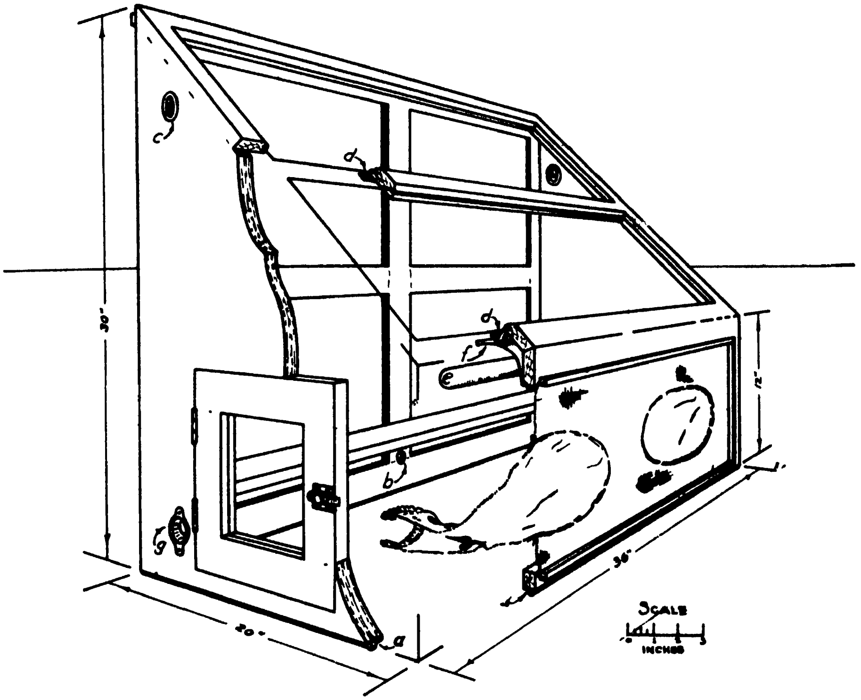
[3] Fawcett, H. S. Citrus diseases and their control. McGraw-Hill Book Co. (New York) 656 pp. (*See* p. 255.) 1936.

[4] Bitancourt, A. A. As doenças de virus dos Citrus. O Biologico 1: 255–262. 1935.

[5] Word has been received from Argentina that mites of the genus *Tennuipalpus* have transmitted the disease known as lepra explosiva, probably the same as leprosis, in northern Argentina.



A hole in the center of the rear base-board (*b*) permits of the introduction of a jet of flowing steam when desired. A hole near the top of each side is fitted with a brass collar (*c*), a fine-mesh screen being soldered to the inside edge and a similar screen soldered to a removable, flanged cover on the outside edge, enabling replacement of a wad of glass wool. A 4" shelf is fastened against the rear, 5" from the bottom. A metal trough (*d*) is tacked at the bottom edge of each pane of the sloping glass top to catch any moisture that may condense on the glass during steaming; this moisture may be withdrawn by removing a strip of absorbent cotton laid in the trough.

Illumination at night is provided by two 11", 40-Watt, showcase bulbs (*e*) screwed into a double-end socket secured to the center of the board at the bottom edge of the top, and backed by a curved, sheet-metal reflector (*f*). The socket is wired to a plug (*g*) sunken in one side near the bottom rear corner, into which is plugged an extension cord equipped with a switch. Illumination during the day is afforded through the glass rear by placing the hood before a window.—JOHN EHRLICH, School of Forestry, University of Idaho, Moscow, Idaho.

Evidence of Resistance in Sweetclover to a Phytophthora Root Rot.[1]—Among the few reports of root rot of sweetclover in the United States, the most extended appears to have been written by Salter and Willard in 1932.[2] From plants dying from this disease in Ohio in the spring of 1937 and 1938, a species of *Phytophthora* has been isolated, which has been identified by Charles Drechsler as *P. megasperma* Drechs.[3] It is believed to be the disease reported in Ohio by Thomas[4] in 1930 and in Kentucky by Valleau in 1932.[5] In 1938 the fungus was found in decaying roots sent from Illinois, and in Wisconsin from as far north as Lake Winnebago. In an earlier year a disease of the same character was seen by the writer in an irrigated sweetclover pasture near Newell, South Dakota, and in roadside plants near Lincoln, Nebraska. Probably the disease is widespread in occurrence, but it appears to be of local economic importance in Ohio, Indiana, and Illinois.

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[1] Contribution from the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, in cooperation with the Wisconsin Agricultural Experiment Station.

The existence of the root rot is first indicated in the spring by the death or unthrifty appearance of individual sweetclover plants. These diseased plants are most evident during hot spring days, when wilting occurs. When the roots of such plants are dug a portion is found decayed. In wet soil the decayed portion is soft and watery with little change of color at first; but, with drying, the diseased area may be shrunken and discolored. The decayed region is usually close beneath the crown, but has been found as much as 8 inches below the crown. Usually, characteristic mycelium is observed in the tissue upon microscopic examination.

In general, the disease is most abundant in low, wet portions of fields, where, during seasons of heavy spring rainfall, it may destroy almost all of the plants quite rapidly. Usually, it has been found occurring in individual plants or in small groups of plants in fields or by roadsides. Plants in vigorous growth and approaching the flowering stage or older have not been found dying of this disease.

Since it appears that Phytophthora root rot may be increasing in severity with the repeated planting of sweetclover, search has been made for resistant plants. Seedlings have not been infected readily. Roots are, however, susceptible in early autumn and thereafter without apparent increase in susceptibility after winter freezing. Moreover, preliminary tests indicate that infection is obtained seemingly in about the same degree in soil temepratures ranging from about 10° to 24° C. Thus, tests for resistance have been made in the following manner for convenience. Roots of plants grown in the field during the summer are dug in late autumn and stored in boxes of sand in a cold frame where the crowns may freeze, though not severely. In January, or at a convenient time thereafter, these roots have been inoculated by inserting mycelium from an agar culture beneath the bark and planted in flats, if convenient. If the roots are large, however, they are packed in sand in baskets and set in the greenhouse at about 65° F. and kept thoroughly wet. In about 3 weeks decay is so well advanced that the roots may then be sorted; those showing no decay are given a second inoculation and set in soil. This second inoculation usually kills but few plants. Surviving plants produce seed in the greenhouse in spring.

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[2] Salter, Robert M., and C. J. Willard. Report on sweetclover research in Ohio. Ohio Agr. Expt. Sta., Dept. of Agronomy, Mimeograph Rept. 16: 124–125. 1932.

[3] Drechsler, Charles. A crown-rot of hollyhocks caused by *Phytophthora megasperma*, n.sp. Jour. Wash. Acad. Sci. 21: 513–526. 1931.

[4] Thomas, R. C. A new disease of sweet clover. Ohio Agr. Exp. Stat. Bull. 446: 72–73. 1930.

[5] Valleau, W. D. Spring dying of sweet clover. In Cooper, Thomas P., Ky. Agr. Exp. Sta. Ann. Rept. 46: 31. 1933.

Thus far all common white sweetclover roots obtained from the roadside or commercial fields have proved susceptible. However, in 1938, occasional resistant plants were obtained from a few selected white sweetclover strains and a foreign selection yielded many of them.

Selfed seed from those resistant selections was planted in the summer of 1938, and some of the progenies have been tested. Thirteen of the larger progenies have now been inoculated and have given 50 to 75 per cent of healthy plants in comparison with 10 per cent healthy plants from the progeny of an unselected sister plant. Thus it appears that resistance to this Phytophthora root rot can be greatly increased by selection.—FRED REUEL JONES, University of Wisconsin, Madison, Wisconsin.

Infectious Variegation of Citrus.—On October 7, 1937, attention was drawn by H. L. Thomason[1] to an effect on lemon leaves of a single tree at Glendora, California, resembling that commonly known as variegation on ornamental plants. Portions of the leaf lacked any green color and were white to yellowish-white. The chlorotic areas varied much in size. In some leaves these areas were mostly on one half of the leaf blade as divided by the midrib. The areas were not arranged in any regular pattern. Buds from this lemon tree were placed in sour-orange stock and in a few months the effect was seen to have passed over into the leaves of the sour-orange stock, producing effects fully as definite as in the lemon leaves. Irregular areas, 5 to 10 mm. or more across, were found on leaves forming subsequently to budding (Fig. 1). When leaves were small and rapidly growing they also showed the flecking characteristic of that associated with psorosis,[2] namely, light-colored, small areas a few mm. in length in the region of the small veinlets. As the leaves matured, some of them became warped and pocketed, suggestive of crinkly leaf of lemon.

In certain general aspects the effects on sour orange have some resemblance to the infectious chlorosis described by Petri.[3]

Lemon buds from another tree at Chula Vista, California, which showed less striking leaf symptoms and was associated with bark symptoms of psorosis on sweet-orange stock, when budded into sour-orange stock, tra[illegible]mitted to this stock an effect similar to that of the infectious chlorosis herein described. It was, however, a less striking and less severe type. This sug-

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[1] Growers' Service, Field Department, Mutual Orange Distributors.

[2] Fawcett, H. S. Is psorosis of citrus a virus disease? Phytopath. 24: 659–667. 1934; *and* Citrus diseases and their control. McGraw-Hill Book Co. (New York). 2nd ed. 656 pp. 1936.

[3] Petri, L. Variegatura infettiva della foglie di *Citrus vulgaris* Risso. Bol. R. Staz. Pat. Veg. (n.s.) 11: 105–114. 1931.

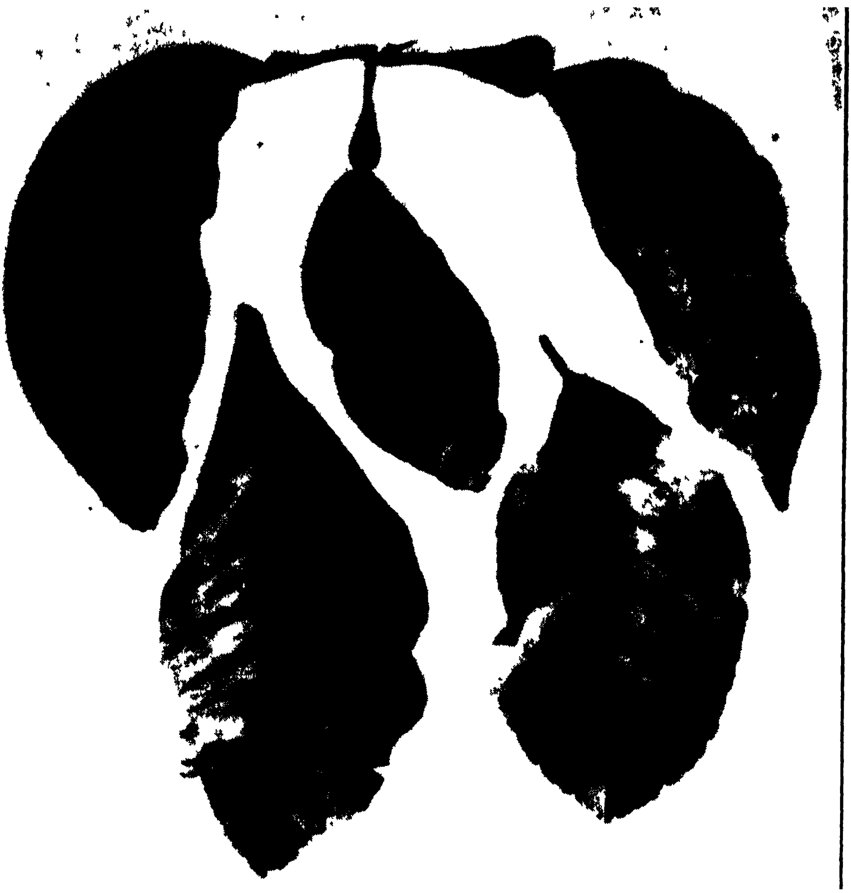

FIG. 1. Infectious variegation of citrus. Two lower leaves show natural infection on lemon; 3 upper leaves show affected sour-orange leaves, the malady being transmitted by budding.

gests a possible relationship to infectious variegation of psorosis in citrus.[4] —H. S. FAWCETT AND L. J. KLOTZ, University of California Citrus Experiment Station, Riverside, California.

Four Fungus Parasites of Sweetclover Infecting Seed.[1]—In a previous note[2] the writer recorded finding the stem-blighting fungus, *Ascochyta caulicola* Laub., infecting sweetclover seed. In the course of searching for this fungus in seed, other species parasitic on this plant were found until at present the entire list of species identified is as follows:

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[4] Fawcett, H. S., and L. J. Klotz. Types and symptoms of psorosis and psorosis-like diseases of citrus. Phytopath. (Abstract) 28: 670. 1938.

[1] Contribution from the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Dept. of Agriculture, in cooperation with the Wisconsin Agricultural Experiment Station.

[2] Jones, Fred Reuel. A seed-borne disease of sweetclover. Phytopath. 28: 661–662. 1938.

Ascochyta caulicola Laub.; *Cercospora zebrina* Pass. (?); *Leptosphaeria pratensis* Sacc. & Briard (*Stagonospora meliloti* (Lasch) Petr.); *Mycosphaerella lethalis* Stone.

After these species were identified from seed lots collected where disease was known to occur, a number of seed samples, sent to the Wisconsin Seed Laboratory for routine analysis, were examined to determine the frequency with which these fungi occur in commercial seed. These samples were presumably grown on Wisconsin farms. Three hundred to 500 seeds were treated 10 minutes in concentrated sulphuric acid, and were washed for 5 minutes in sodium hypochlorite solution of approximately .4 per cent concentration. The seed was then plated on potato-dextrose agar. After 5 to 7 days the fungi on germinating seed were identified or transferred.

Of the 27 samples examined, *Cercospora* was found in 13, *Ascochyta caulicola* in 6, and *Leptosphaeria pratensis* in 6. Inasmuch as the efficiency of the method used in plating these fungi has not been tested, the percentage of living seed of any single sample carrying these fungi cannot be accurately stated. However, *Cercospora* was found in more than 1 per cent of the seed in some samples; but, the other above-named species were obtained from less than 1 per cent of the seed. No external evidences of the presence of these fungi in the seed were discovered. *Cercospora* fruited abundantly on the discarded seed coat in 5 to 7 days at room temperature. None of the fungi immediately attacked or damaged the seedlings bearing them. *Mycosphaerella lethalis* was not found in these commercial lots, but it was isolated from seed of *Melilotus dentata* grown in an experimental plot where the plants were severely attacked by this fungus.

The extent to which this seed infection leads to the incidence of important diseases of sweetclover in agriculture remains to be determined. That *Ascochyta caulicola* is distributed chiefly in seed seems likely from evidence given previously, but evidence in the case of the other 3 parasites is lacking. Such evidence may well be sought, however, in investigations directed toward the control of the diseases caused by these fungi.—FRED REUEL JONES, University of Wisconsin, Madison, Wisconsin.

Chlorine Gas as a Seed Disinfectant.—Chlorine gas has been advocated and is being employed commercially to some extent as a seed disinfectant. A patent on its use for this purpose and on a device for its application to seed was applied for in 1937. This chlorine-gas treatment is claimed to be superior to other treatments in the following respects: (1) It is much cheaper; (2) it does not render the seed unfit for feed; (3) it improves germination and stand; (4) it controls certain smuts and other seed-borne diseases; (5) it kills weevils and other insects in the seed; and (6) it does not injure bags, drills, or operators. Because of these claims and numerous inquiries regarding the merits of chlorine as a fungicide, its usefulness in this field was investigated jointly by the Bureau of Plant Industry and the Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture, during the period from 1937 to 1939.

In laboratory experiments, exposure for 5 minutes to concentrations of 50 to 100 per cent chlorine gas by volume failed to kill all the spores of barley covered smut and sorghum covered kernel smut, but exposure for 2 hours to a concentration of only 1 per cent, under certain conditions, completely inhibited spore germination in these smuts.

Bunt in wheat was adequately controlled by exposing the seed for 2 hours to a concentration of 3 to 9 per cent chlorine gas; covered kernel smut in sorghum was controlled by exposure to 10 per cent chlorine gas for 1 hour. Smuts of oats and barley were not appreciably affected by exposure of the seed to a concentration of 10 per cent chlorine gas for 2 hours.

In general, it was found that to secure satisfactory killing of smut spores borne on the surface of the seed without causing pronounced seed injury, the gas concentration should not be less than 3 or greater than 9 per cent, the time of exposure should be from 1 to 2 hours, and the volume of pure chlorine gas used should not be less than 20 per cent nor more than 40 per cent of the net volume of the seed being treated.

The device, designed and used for treating seed with chlorine gas, was observed while it was in operation. The treatment consisted of exposing the seed to a concentration of less than 0.5 per cent chlorine gas for a period of about 3 minutes. Several test lots of smut infested seed of wheat, oats, and barley were thus treated in this device and sown on the Arlington Experiment Farm near Washington, D. C. In no case was improvement in emergence or stand observed or satisfactory smut control obtained.

In experiments with weevils it was found that some active adult grain weevils survived after exposure to concentration of 10, 20, and 50 per cent chlorine gas for 60, 20, and 10 minutes, respectively.

While chlorine gas may effectively control certain seed-borne diseases, if applied for a sufficiently long time at the proper concentration, it is not adaptable to the continuous type of seed treatment equipment now in common use, but calls for an air-tight "batch" type of treater. In this the seed must remain exposed to the gas for one to two hours, thus considerably slowing up the work of treatment. The chlorine treatment may be found suitable for treating the kinds of seed of which relatively small quantities are required for large acreages.

This note is a summary of a more complete report being submitted for publication as a U. S. Department of Agriculture Circular.—R. W. LEUKEL, Division of Cereal Crops and Diseases, Bureau of Plant Industry, and O. A. NELSON, Division of Insecticide Investigations, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture.

BOOK REVIEWS

Wormald, H. *Diseases of Fruits and Hops.* 290 p., 24 figs., 40 plates. 17s 6d net. Crosby Lockwood & Son, Ltd. (London). 1939.

Dr. Wormald, assistant director and for many years plant pathologist at the East Malling Research Station, has written this book primarily for commercial growers, gardeners, students, and advisers in horticulture. "The object of this book is to enable the grower to recognize the various disorders that affect his fruit and hops, and to provide information that will help him to control them. It, therefore, aims at describing symptoms rather than organisms causing the disease, but brief descriptions of the parasites and their habits are given so that the measures advocated may be understood. . . . No attempt has been made to give a complete list of references to papers on each subject, and only those published in Britain, or dealing with diseases as occurring in the British Isles, are quoted." From the American point of view these limitations greatly lessen the usefulness of the book, however desirable they may be from the viewpoint of the fruit growers of Britain.

In chapter I, dealing with factors conducive to health or disease in plants, the following are discussed briefly in order, under the heading "Fruit Trees as Living Organisms": Physiological disturbances in the absence of parasites, mechanical injuries, infection, fungi, bacteria, viruses; and under the heading "Factors Underlying Incidence of Disease": The presence of the parasite, the susceptibility of the host plant, environmental conditions, and man's efforts to control disease. Chapter II is a discussion of the commonly used copper and sulphur fungicides and their application as sprays and dusts. Chapters III–XIV are brief, but accurate and concise, descriptions of diseases of apple, pear, quince, medlar, plum, peach, nectarine, apricot, cherry, currant, gooseberry, raspberry, loganberry, blackberry, strawberry, grape, fig, mulberry, walnut, and hops, with life histories of the pathogens frequently outlined and often with text figures showing spore forms. The descriptions are supplemented by 40 plates in half-tone that are beyond any praise that this reviewer is capable of giving. They set a new standard in illustrations for books on plant diseases. A unique and effective method of treatment is the grouping of certain diseases that attack a number of different plants. Chapter III is devoted to general descriptions of these diseases, although they are mentioned again under specific hosts with special reference to particular symptoms or injuries.

Chapter XV, the final chapter, deals with important diseases not yet recorded in Britain. These are nearly all of American origin and include brown rot, cherry leaf spot, plum and cherry black knot, fire blight, bacterial spot of stone fruits, peach yellows and other virus diseases, and the following diseases affecting chiefly the apple: anthracnose, perennial canker, blister canker, blotch, and internal cork. The book is well indexed and appears free from typographical errors. The printing and general format are excellent. The text throughout is written with the clearness and preciseness that characterize all of Dr. Wormald's publications.

Specialists in fruit and hop diseases in all countries of the temperate zones will find this book, with its concise but complete descriptions and its fine illustrations, a valuable reference book, although it would be much more useful if it included references to the literature of countries other than Britain. To fruit growers in Britain the book should be indispensable.—**John W. Roberts.** Bur. Pl. Ind., Horticultural Station, Beltsville, Md.

Wolf, Frederick A. with the collaboration of **K. H. Garren** and **J. K. Miller.** *Fungi of the Duke Forest and their relation to Forest Pathology.* Duke University School of Forestry Bulletin 2. 122 pp. 53 text figs. (Durham). 1938. $1.00.

The author and his associates, over a period of six years, have been collecting and cataloguing the fungi of the Duke Forest as a basis for future research in forest pathology. A chapter outlining and discussing in general the larger fungus groups encountered in the work is followed by a more detailed consideration of the more important fungi found causing tree diseases and decays in the forest. This material is arranged topically under the host involved, and many of the fungi discussed are illustrated. A complete list of all fungi collected and determined follows, together with a host index.—**John A. Stevenson,** Bureau of Plant Industry, Washington, D. C.

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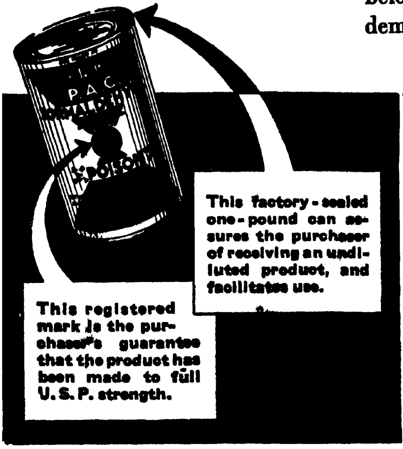

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A STUDY OF VIRUSES CAUSING YELLOW MOSAICS IN EUROPEAN AND AMERICAN VARIETIES OF THE POTATO, SOLANUM TUBEROSUM

T. P. DYKSTRA[1]

(Accepted for publication June 1, 1939)

INTRODUCTION

Among the different viroses found on the potato are the so-called yellow mosaics. The symptoms caused by the viruses of this group on some of the potato varieties consist of regular or irregular leaf blotches. These are devoid of chlorophyll and assume a bright, yellowish-white or gray color. Some of these viroses cause an internal necrosis in tubers of affected plants. Aucuba mosaic, pseudo-net necrosis, tuber blotch, and calico have been thought to belong to this group. Whereas calico is found in this country, the other diseases presumably are confined to Europe and have been studied extensively in The Netherlands and Ireland (20, 1, 9, 4). The objects of the investigations reported in this paper were to determine (1) whether aucuba mosaic, pseudo-net necrosis, and tuber blotch corresponded to viroses found on potatoes in this country, and (2) whether there was a natural relationship between the different viruses causing the yellow mosaics, or whether they were distinct and unrelated.

Although no one criterion is available to determine definitely strain relationship between different viruses, when a number of tests, such as (a) serological reactions (2, 3); (b) protective inoculation (10, 16, 19, 23, 24, 26); (c) determination of physical properties, *i.e.*, thermal inactivation point, longevity of the virus *in vitro*, and (d) symptoms and general behavior of the disease, are applied, fairly definite information in regard to strain relationship may be obtained. In the present paper these means have been used in the study of the different viroses.

A new and undescribed disease, tentatively designated Canada streak, has been included also in these studies because it was found attributable to a closely related strain of the aucuba-mosaic virus.

These experiments are a continuation of the studies on American and European potato viroses already reported (5, 6, 7, 8).

MATERIAL AND METHODS

In 1936, some tubers infected with pseudo-net necrosis were received from H. M. Quanjer of the Agricultural College, Wageningen, The Netherlands. At the same time the blotch virus and interveinal mosaic in President were received from P. A. Murphy of Glasnevin Agricultural College, Dublin, Ireland. In 1934, a tuber of the variety Irish Daisy was received from D. H. Putnam of Toronto, Canada. This tuber was afflicted with a virosis tentatively designated as Canada streak. Tubers affected with aucuba mosaic, and with calico were from our regular stock cultures of potato viruses, and had been propagated under insect-proof cages for the last 10 years.

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[1] Pathologist, Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

Since all the viruses used in these studies can be transmitted by juice transfer, the leaf-rubbing method, involving carborundum dust as an abrasive, was employed in most cases, although occasionally stem grafts were made. All the viruses used were in biologically pure form, *i.e.,* any one virus studied was present in plants free from contamination of other viruses, including X.

STUDIES ON INDIVIDUAL VIROSES

The symptoms described were those observed under greenhouse conditions at temperatures varying from 65° to 75° F.

Aucuba Mosaic

Quanjer (20) was the first to describe the aucuba mosaic of potato plants and so named it on account of its resemblance to the natural mottling of *Aucuba japonica.* The virus causing this disease has been referred to as G by Clinch *et al.* (4), and as Solanum virus 9 by Smith (25). This disease is distinct from the aucuba mosaic of the tomato, caused by a virus closely related to the common tobacco virus. In 1926, Atanasoff (1) stated that net necrosis was a tuber symptom of aucuba mosaic, in which the parenchymatous tissues of the tuber become necrotic, but in which the vascular tissue is not involved. In 1929, Elze and Quanjer (9) concluded that Atanasoff had been dealing with a mixture of aucuba mosaic and pseudo-net necrosis and that he was incorrect in regarding the latter as a symptom of aucuba mosaic.

The symptoms of this disease are similar in most of the different American and European potato varieties tested, and consist of small, round, bright-yellow spots about 4 mm. in diameter, sometimes coalescing to form large yellow patches. In the European variety, British Queen, foliar necrosis and a wilting of the leaves develop in addition to a pronounced yellow mottling. These reactions also have been reported by Clinch *et al.* (4), who found that the presence of virus A caused in potato foliage an exaggeration of the mottle. The writer did not find this true of the variety Green Mountain and Seedling 41956. These two varieties, infected with virus A, were inoculated with the aucuba-mosaic virus; but the intensity of the chlorotic spots of the leaves did not differ from that shown by the same varieties affected only with aucuba mosaic. Clinch *et al.* (4) found tuber necrosis in the parenchymatous cells of both cortex and pith. They found it externally visible as irregular brown patches in 7 of the 14 varieties examined, although in some cases it was very mild. The writer failed to find any necrosis in tubers of the following varieties: Seedling 41956, Katahdin, Chippewa, Green Mountain, Bliss Triumph, and Irish Cobbler. The investigations on this disease included also symptom studies on other solanaceous plants. Quanjer (20) stated that tobacco was a symptomless carrier of this virus and Clinch *et al.*

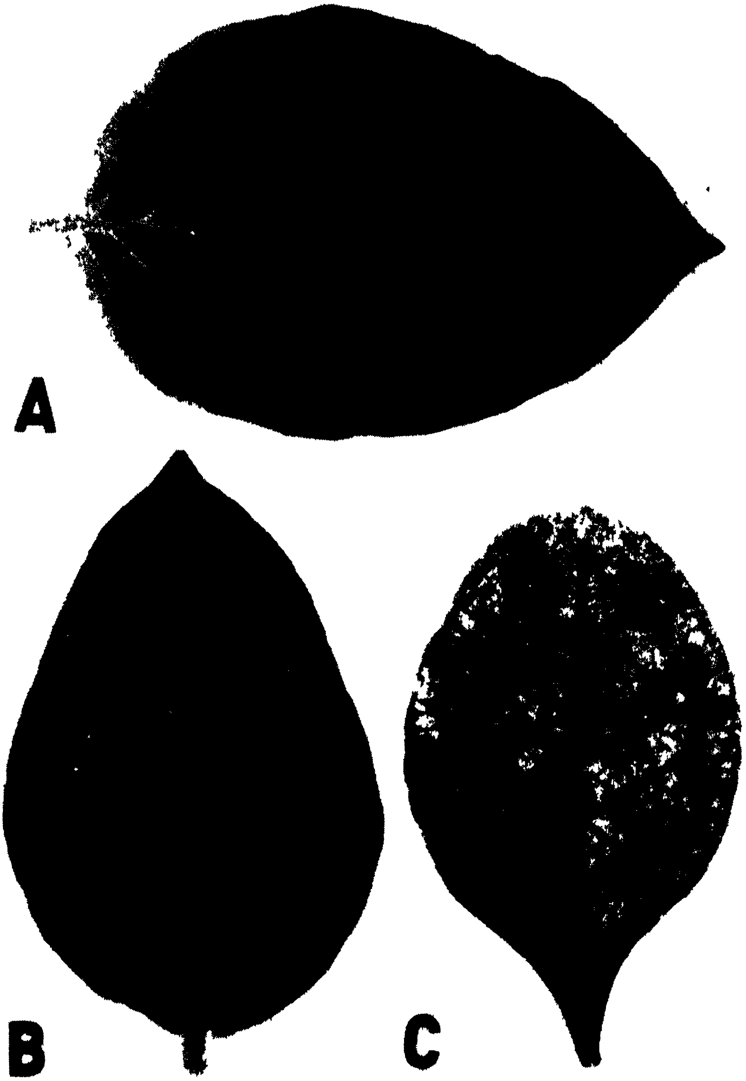

FIG. 1. Leaves of *Nicotiana tabacum* showing: A. Blotchy type of mottling due to infection of the aucuba mosaic virus free from virus X; B. An irregular blotchy type of mottling due to the tuber blotch virus; and C. Symptoms of Canada streak free from virus X.

(4), also, state that tobacco is readily inoculable but shows no symptoms. The writer's findings do not agree with theirs, since irregular yellow blotches on the upper half of the infected tobacco leaves were generally observed (Fig. 1, A). Symptoms somewhat similar to those found in tobacco, but more uniformly distributed over the entire leaf, were found in *Nicotiana glutinosa.* In *N. sylvestris* a few scattered semi-dried spots developed on the leaf blades. Infected pepper plants developed necrotic spots in the top

TABLE 1.—*A summary of comparison of the properties of the aucuba-mosaic group of viruses on Nicotiana sylvestris as the host*

Viruses derived from	Thermal inactivation point °C.	Number infected[a]	Tolerance to dilution	Number infected[a]	Longevity *in vitro* 1 to 10 at 15° C.	Number infected[a]	Resistance to pH, using disodium-phosphate-citric acid buffer, after 24 hours	Number infected[a]
					hours			
Aucuba	60	10/10	1–10	10/8	24	10/9	2.2	10/0
	62	10/10	1–100	10/6	48	10/8	3.8	10/0
	65	10/7	1–500	10/2	72	10/5	4.2	10/2
	68	10/0	1–1000	10/0	96	10/1	4.7	10/6
	Nontreated	10/10					6.0	10/6
							8.5	10/2
Canada streak	60	10/8	1–10	10/10	24	10/10	2.2	10/0
	62	10/10	1–100	10/10	48	10/8	3.8	10/0
	65	10/6	1–500	10/4	72	10/6	4.2	10/3
	68	10/0	1–1000	10/0	96	10/5	4.7	10/3
	Nontreated	10/10					6.0	10/5
							8.5	10/1
Pseudo-net necrosis	60	10/6	1–10	10/8	24	10/8	2.2	10/0
	62	10/7	1–100	10/5	48	10/7	3.8	10/0
	65	10/0	1–500	10/0	72	10/4	4.2	10/1
	68	10/0	1–1000	10/0	96	10/1	4.7	10/3
	Nontreated	10/6					6.0	10/4
							8.5	10/0
Tuber blotch	60	10/7	1–10	10/7	24	10/8	2.2	10/0
	62	10/5	1–100	10/6	48	10/6	3.8	10/1
	65	10/0	1–500	10/0	72	10/3	4.2	10/0
	68	10/0	1–1000	10/0	96	10/0	4.7	10/3
	Nontreated	10/7		10/0			6.0	10/4
							8.5	10/1

[a] Numerator, number of plants inoculated. Denominator, number of plants infected.

leaves. The leaves finally withered and, under relatively high air temperature conditions, 75° F. and above, the plants died about 20 days after inoculation. No symptoms could be detected following inoculation of Petunia, but no return inoculations were made to potato to determine whether this plant is a masked carrier.

This virus can be diluted 1–500 and still cause infection; its thermal inactivation point is between 65° and 68° C., and it can exist *in vitro* for 96 hours (Table 1). This confirms the results of Clinch *et al.* (4).

Canada Streak

In the spring of 1934, a tuber of Irish Daisy (Fig. 3, C), infected with an unknown virus, was received from D. H. Putnam of the University of Toronto, who obtained it originally from Nova Scotia. It was found readily transmissible to different potato varieties by juice transfer, which generally resulted in 100 per cent infection. Symptoms developed within 2 to 3 weeks and varied somewhat with different varieties. In the virus X, immune seed-

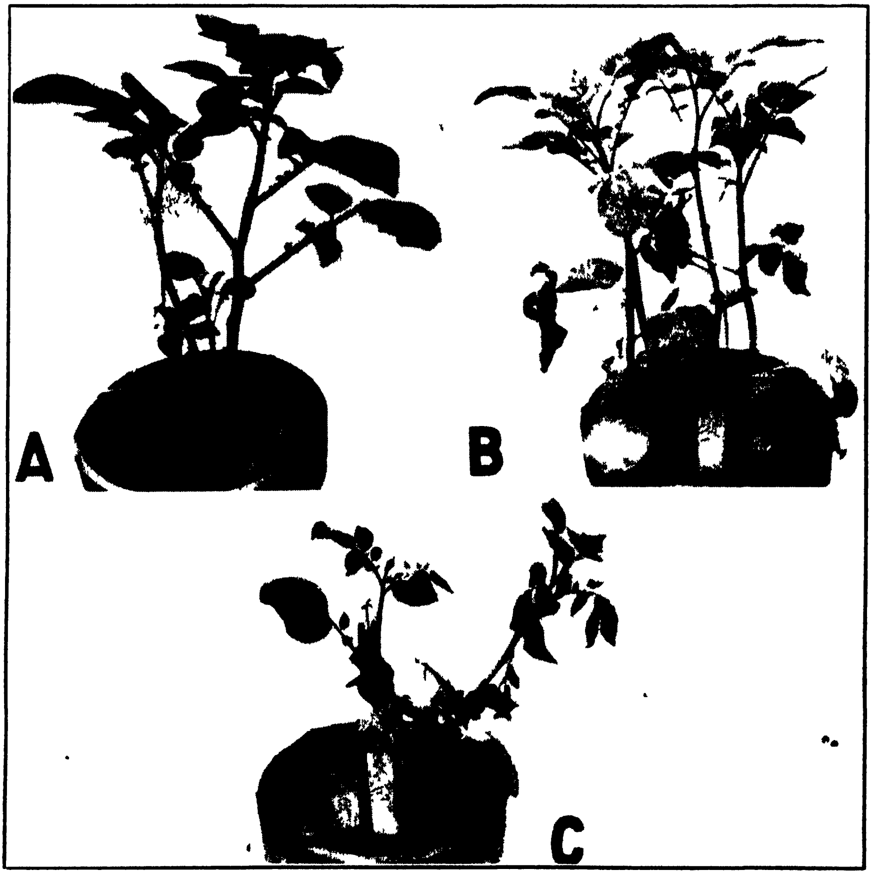

FIG. 2. Canada streak symptoms on the following potato varieties: A. Bliss Triumph; B. Early Rose; and C. Arran Chief.

ling variety 41956, a blotchy mottling of the lower leaves resembling aucuba-like symptoms has been noted. In other cases stem necrosis, a burning of the veins and petioles, has been observed in the same variety. When juice from chlorotic plants (Fig. 3, A) was transferred to Bliss Triumph plants, considerable leaf necrosis developed similar to that which occurred when they were inoculated with juice from necrotic plants (Fig. 2, A). In the early stages, second-generation symptoms of Earliest of All plants, infected with Canada streak, were manifested by a rugosity of the leaves, which had a tendency to roll downward, with the veins of the lower leaves becoming necrotic. At this stage the disease could be mistaken easily for rugose mosaic. When the plants grew larger necrotic spots appeared on the foliage resembling "early-blight" infection, except that concentric rings were absent. In addition some yellow blotches were evident.

The symptoms on Green Mountain plants corresponded in general to those found on the variety Earliest of All. In Irish Cobbler the lower leaves were fairly well covered with necrotic areas and soon dropped. Yellow blotches were evident in the top leaves. Considerable necrosis was prevalent in the stem and extended into the cortex and pith. In Chippewa, necrotic dried areas were found on the intermediate leaves, but almost no aucuba-like symptoms were evident. In President necrotic spots were found on the lower and intermediate leaves, whereas typical aucuba-like symptoms developed on the top foliage. In Epicure necrotic blotches and streak symptoms were essentially absent. The lower leaves were chlorotic, with just a little of the green interspersed. Infected Arran Victory plants showed considerable of the aucuba-like symptoms in addition to the necrotic spots on the lower and intermediate leaves. The injury on the European variety Arran Chief (Fig. 2, C, and 3, B) was especially severe, resulting in a scorching

FIG. 3. Canada streak symptoms on leaflets of the following potato varieties: A. Bliss Triumph showing aucuba type of mottling; intermediate leaves on the same plant showed considerable necrosis; B. Arran Chief; C. Irish Daisy.

and finally causing almost every leaf on the plant to drop. The tubers of every variety tested showed an internal blotchy necrosis, starting in the pith, often at the stem end and in severe cases, spreading through the entire tuber. The intensity of the necrosis varied somewhat depending upon the variety (Fig. 4). The tuber symptoms generally did not become evident until about 2 months after harvesting.

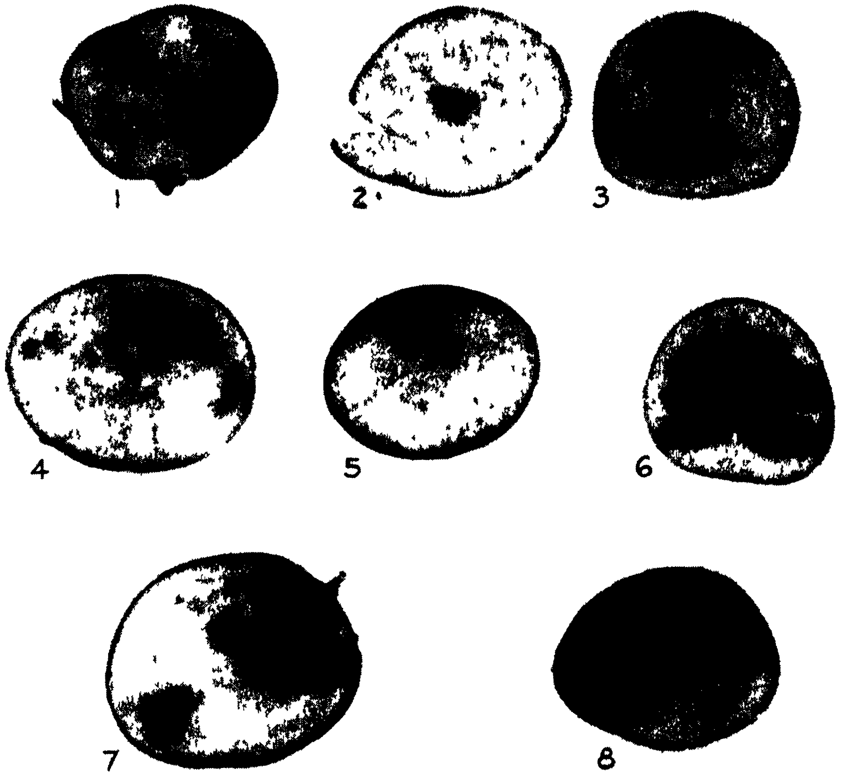

FIG. 4. Internal symptoms of tubers of different varieties infected with Canada streak virus. Note that the parenchyma tissue rather than the vascular region is affected. The slices were cut from infected tubers of the following varieties: 1, Seedling 41956; 2, Sebago; 3, Irish Cobbler, 4, President; 5, Arran Victory, 6, Chippewa; 7, Green Mountain, 8, Bliss Triumph.

Tobacco plants, inoculated with this virus, developed irregular white blotches on the upper half of the leaves (Fig. 1, C) The leaves of infected young pepper plants developed necrotic blotches, stems became necrotic, also; and within 20 days after inoculation the plants were dead. This was not true of the older infected plants; these developed necrotic leaves that later dropped, but the plants were not killed. In *Nicotiana glutinosa* brownish, dried areas appeared on the intermediate leaves. The top leaves developed a typical aucuba type of mottling. In *N. sylvestris* dried spots, about 2 mm. in diameter, developed on most of the leaves in addition to small circular yellow spots

The yellow blotches on the foliage of different solanaceous plants, which, in addition to necrosis, were generally evident, suggested a possible relationship between this disease and aucuba mosaic. Through the courtesy of K. Starr Chester, the viruses causing the above-mentioned diseases were subjected to a serological test. In a letter dated May 29, 1937, he stated: "The close relationship of Canada streak to potato aucuba mosaic has been repeatedly confirmed." This indicated relationship, but it did not exclude the possibility that Canada streak might result from a complex, comprising, perhaps, a combination of aucuba mosaic and tuber blotch, the latter being responsible for the necrotic spots on the foliage and the internal brown blotches of the tubers.

A number of experiments were designed to demonstrate whether Canada streak is induced by one or more than one virus. Accordingly, inoculations were made into young potato plants of British Queen, Green Mountain, Bliss Triumph, and Irish Cobbler; the sources of inoculum were juice extracts from potatoes affected with (a) Canada streak, (b) aucuba mosaic, and (c) tuber blotch. In addition to these 3 sources of inoculum, a mixture of equal parts of juice taken from aucuba mosaic and tuber-blotch-infected plants was used. Canada streak, aucuba mosaic, and a mixture of aucuba mosaic and tuber blotch caused a foliar necrosis and also bright chlorotic patches on the leaves of British Queen, whereas tuber blotch alone developed only necrotic blotches on the leaves, without any chlorosis. In Irish Cobbler and Green Mountain, the combination of aucuba mosaic and tuber blotch produced, in addition to aucuba-mosaic symptoms, a slight foliar necrosis caused by tuber blotch, and resembled somewhat the symptoms caused by the Canada streak virus in these two varieties. In Bliss Triumph the combination of tuber blotch and aucuba mosaic failed to develop any necrosis, whereas foliar necrosis, in this variety, is always rather pronounced when the host is affected with Canada streak (Fig. 2, A).

Several attempts were made to separate different viruses from the chlorotic and the necrotic spots of infected *Nicotiana sylvestris* plants. Small amounts of tissue were carefully removed from chlorotic spots and used to inoculate healthy *N. sylvestris.* Tissue taken from necrotic areas also was used as source of inoculum, but, in all instances, Canada-streak symptoms developed consisting of both chlorotic and necrotic spots.

In transmission studies of tuber blotch to other solanaceous plants, it was found that, ordinarily, smaller percentages of infection took place than when Canada streak was used as the source of inoculum. In the several hundred inoculations made with the last-named virus into several different host plants, the Canada-streak symptoms were always observed. This was true when property studies of this virus were carried out and at times only a small percentage of infection was secured. If this disease is induced by a virus complex consisting of more than one component, it is believed that occasionally one of the components would fail to be transmitted and that this fact would be evidenced by the type of symptoms produced.

All the available evidence supports the fact that Canada streak is caused by a single virus, a strain of aucuba mosaic that also produces necrosis. For physical properties, see table 1.

Experiments were carried out in 1935 to determine if this virus could be transmitted by aphids. About 20 aphids taken from caged potato plants affected with Canada streak were transferred to each of 77 sprouted potato seed pieces. Forty-seven seed pieces were colonized with *Myzus circumflexus*, Buckl., 18 with *Myzus pelargonii*, Kalt., and 12 with *Macrosiphum solanifolii* (Ashm.). After the aphids had fed on the tender sprouts for 6 days, the seed pieces were fumigated and planted in the field. The plants were kept under observation until maturity, but in no case were disease symptoms observed.

Pseudo-net Necrosis or Tuber Blotch

This virus was designated as F by Clinch *et al.* (4) and as Solanum virus 8 by Smith (25). Clinch *et al.* (4) came to the conclusion in 1936 that these two diseases most probably are caused by a similar virus. The writer started his studies by treating these two diseases as if caused by two distinct viruses; but, as the work progressed, the similarity of pseudo-net necrosis and tuber blotch became evident.

Quanjer, Thung, and Elze (21), in 1929, first described pseudo-net necrosis. They found the symptoms on potato consisting of necrotic spots in the storage parenchyma next to the external and internal phloem of the tubers. They stated that pseudo-net necrosis developed during storage and that its development was accelerated by a rise in temperature. They stated also that this disease had been observed by them in Roode Star and several other varieties, unaccompanied by foliage symptoms. They found that it spread under field conditions, and succeeded in transmitting it by means of *Myzus persicae*.

The writer has transmitted this virus by leaf rubbing to a number of potato varieties, such as Bliss Triumph, Irish Cobbler, Chippewa, Katahdin, Green Mountain seedlings, and to some numbered seedlings of miscellaneous varieties. In most of the seedling varieties necrotic blotches developed on the intermediate leaves. Small yellow blotches were evident on the leaves of infected Bliss Triumph. In Katahdin and Irish Cobbler a very pronounced necrosis of the tissues between the veins of the leaf blade developed, and some necrosis was found on the midribs and smaller veins. The intermediate leaves turned yellow and eventually dropped.

Necrotic blotchy spots have been found in tubers of infected plants of the following varieties: President, Bliss Triumph, Irish Cobbler, Katahdin, Chippewa, and Seedling 41956. The intensity of the tuber necrosis generally was not so severe as in the case of Canada streak in the same variety. The pseudo-net-necrosis virus was also studied on other solanaceous plants. In *Nicotiana glutinosa* yellow, aucuba-like symptoms were produced quite similar to those developing in tobacco (Fig. 1, B) and consisted of irregular yellow patches on the leaf. In *N. sylvestris* distinct light necrotic blotches developed in the tissue between the veins of the leaf blade, giving the plant a rusty appearance. The symptoms in pepper plants consisted of longitudinal streaking of the stem and necrotic spots on the top leaves, which finally dried and hung on the stem for a time by means of their dried petioles. Eventually, every leaf of the plant dried and dropped.

Ten President plants infected with this disease also were inoculated with Canada streak. Although the plants were kept under observation for 2½ months, they failed to develop any additional symptoms. At the end of that period, one leaf from each of the ten plants was taken, the juice extracted and inoculated into 10 plants each of potato and *Nicotiana sylvestris.* Only pseudo-net necrosis symptoms developed, indicating that the presence of this virus in the plant protected it against Canada streak. This would indicate that these two viruses are closely related, as it is considered that the existence of a virus in a plant protects it only against infection of other strains of the same virus. When five healthy President plants were inoculated at the same time with the Canada-streak virus, every one of these developed severe foliar necrosis within 3 weeks after inoculation. For property studies see table 1.

In 1933 Loughnane and Clinch (13) first described tuber blotch as a new disease. They isolated it from Glasnevin stock harboring interveinal mosaic, which, according to these authors, is caused by a combination of tuber blotch and virus X. Tuber blotch was so named because of a blotchy type of necrosis in tubers of affected plants.

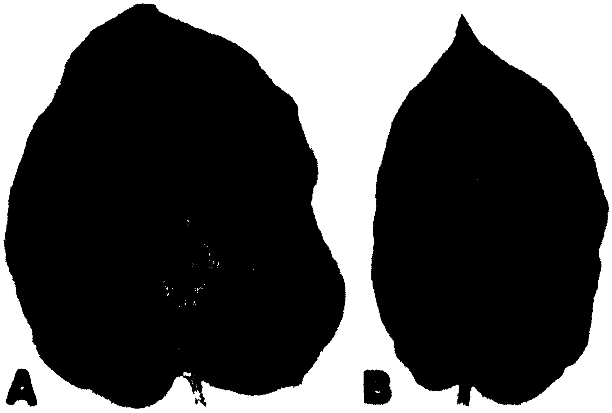

FIG. 5. Symptoms of tuber blotch on leaflets of A. Irish Cobbler; B. Green Mountain seedling.

They failed to transmit tuber blotch by allowing *Myzus persicae* to migrate from the sprouts of infected tubers to healthy President tubers in the same dish during a period of about 30 days. In one experiment, virus A was present as the source, as well as tuber blotch in the same tuber, with the result that most of the plants became infected with virus A, and a small proportion also produced tuber blotch. They found that a heavy infestation of aphids gave the best results in transmitting tuber blotch. The writer has transmitted this virus by leaf mutilation to a number of potato varieties, namely, Green Mountain, Irish Cobbler, Bliss Triumph, Katahdin, and some miscellaneous seedlings. In some of these seedlings necrotic blotches developed on the intermediate leaves. Katahdin also developed severe necrosis of the leaf blade from infection with the tuber-blotch virus. In seedling 41956, a faint aucuba-like chlorosis developed and similar symptoms were observed in Bliss Triumph. In Irish Cobbler and Green Mountain a foliar necrosis instead of chlorotic blotches developed (Fig. 5). The tuber symptoms were very similar to those caused by pseudo-net necrosis in the same varieties.

In *Nicotiana glutinosa* whitish aucuba-like symptoms occurred that were quite similar to those found in tobacco infected with the same disease. In *Nicotiana sylvestris* distinct light necrotic areas developed in the tissue between the veins of the leaf blade, giving the plant a rusty appearance. The symptoms in pepper plants consisted of longitudinal streaking of the stem and necrotic spots on the top leaves, which finally dried and hung to the stems by their dried petioles. Eventually every leaf of the plant dried and dropped. Clinch *et al.* (4) claim that the Irish interveinal mosaic, attributable to tuber-blotch virus combined with virus X, causes in President (and other varieties) a certain amount of rusty necrosis in the lower leaves in addition to a dark and light green mottling all over the plant, and a necrosis in the tubers. They state that the interveinal mosaic of Irish origin and the corresponding Dutch disease received from Prof. Quanjer in 1925 showed the same symptoms, but the name interveinal mosaic is now sometimes used in Holland in a different sense. Koch and Johnson (12) reported the isolation of a virus from interveinal mosaic received from Ireland, which they called the "potato streak virus." They state: "Primary infection causes distinct necrotic, streak-like symptoms on leaf veins, lamina and stems of potato (Bliss Triumph and Green Mountain varieties) generally resulting in killing of the bud first, followed by a downward necrosis which may kill the entire plant." Illustrations of infected plants show severe top necrosis. These symptoms are quite different from those found in the writer's experiments when the same varieties were affected with tuber blotch. Clinch *et al.* (4) refer to Koch and Johnson's findings as follows: "Although these varieties (Bliss Triumph and Green Mountain) had not been used here at that time, it was considered likely for reasons which were given, that the virus in question was probably identical with the tuber blotch virus, but during the past year additional facts have come to light which complicate the issue and render its identity uncertain."

We have received also an interveinal mosaic-diseased tuber from Ireland, and, although the plant grown from this tuber developed a certain amount of rusty necrosis in the leaf, there was no evidence of light and green mottling all over the plant. All attempts to isolate tuber blotch from this virus complex by transmitting it to virus X-resistant seedling variety 41956 failed, whereas this variety is ordinarily easily infected with the tuber-blotch virus. Potato plants inoculated with interveinal-mosaic virus never developed tuber-blotch-like foliar or tuber symptoms.

Since the tuber affected with interveinal mosaic, originally received from Ireland, showed no tuber necrosis, it is possible that, inadvertently, a tuber was sent not affected with this disease. Some of the President plants infected with tuber-blotch virus were inoculated with the virus X, but the mottling produced appeared not to be more prominent than the mottling that developed when the virus X was introduced into healthy President plants. Different American varieties, already carrying virus X, did not produce an interveinal mottling when subsequently infected with the tuber-blotch virus, either as current-season, or as second-generation symptoms.

Although the writer has no intention on the basis of his experiments to question the findings of Clinch and co-workers on the identity of the Irish interveinal mosaic, he believes it may be of interest to report his observations on this virus complex in this country.

To determine whether tuber blotch is related to Canada streak, 10 President plants showing tuber blotch were inoculated with juice extracted from a potato plant affected with Canada streak. The inoculated plants were kept under observation for 2½ months, but failed to develop any additional symptoms. At the end of that period, 1 leaf from each of the 10 plants was taken, and the juice was extracted and inoculated into 10 plants each of potato and *Nicotiana sylvestris*. As in the case of pseudo-net necrosis, only tuber-blotch symptoms developed. Five healthy President plants were inoculated with Canada-streak virus and developed severe foliar necrosis within 3 weeks after inoculation. These results indicated a relationship between Canada streak and tuber blotch.

In a letter dated July 6, 1937, K. Starr Chester stated in a report of his serological studies on these viruses: "I repeated the test of tuber blotch and pseudo net necrosis against Canada streak and aucuba mosaic several times but was unable to demonstrate any inter-reaction. In this type of work, however, I do not take negative reactions too seriously, so long as the tests of blotch and pseudo net necrosis have not been performed." Chester at that time had resigned from the Rockefeller Institute and further serological studies on these viruses had to be discontinued (Table 1).

Calico Solanum Virus 10 (25)

This disease was first described from Idaho by Hungerford (11) in 1920. He stated that "all evidence to date seems to show that this condition is tuber perpetuated but not infectious." Young and Morris (27) were unable to transmit calico to healthy potatoes by means of core grafts in twenty-two trials. They state that it may be a genetic abnormality instead of a disease. There is a variety known as Posey, which has a variegation closely resembling calico symptoms, but is nontransmissible and is traceable to a heritable genetic abnormality. In a cross between Posey and

Katahdin, in the F_1 progeny of 70 plants, 4 of these showed the variegated condition.[2] It is, therefore, possible that Young and Morris were dealing with a genetic character. McKay and Dykstra (14, 15) found some evidence that this is a virosis, since in two tests calico was apparently transmitted by inoculations. It remained for Porter (17, 18) to definitely demonstrate that calico is an infectious disease and can be transmitted by juice transfer and by aphids, although it was with difficulty transmitted by core grafts.

Calico is characterized by the occurrence of large irregular yellow to cream-color spots on the leaves. In some cases as much as 70 per cent of the leaf surface may be entirely lacking in chlorophyll; then, again, only occasional leaves may show a few spots. Generally, however, the spots are numerous and well-distributed over the plant.

This disease has been studied on White Rose, Burbank, Green Mountain, Bliss Triumph, Katahdin, and on virus X-resistant seedlings. The general foliar symptoms on the different varieties were very similar, and no indications of necrosis were observed in either the foliage or tubers.

Porter (18) carried on experiments to determine the host range of the calico virus and to study the symptoms of the disease on different solanaceous hosts. He used calico on White Rose as the source of inoculum; but, since this variety also carries the virus X, it complicated his symptom studies, for these consisted generally of a combination of both the calico and virus X. It did not, however, interfere with his host-range determination, since return inoculations from tested plants were always made to potatoes, and only when they developed the disease was the plant considered a host. He found that tomato, pepper, *Datura stramonium, Solanum melongena,* and *Petunia* spp. were hosts of the calico virus, but failed to transmit and recover it from *Nicotiana tabacum.*

The writer has studied this virus on other solanaceous plants, and eliminated the virus X, by transmitting calico from Green Mountain to virus X-resistant seedling 41956, thereby securing calico in biologically pure form.

In *Nicotiana sylvestris* a very pronounced yellow blotchy mottling developed on the leaves, and the yellowish patches had a slight tendency to dry, giving the plants, in addition to the mottling, a somewhat rusty appearance. In *Nicotiana glutinosa* irregular-shaped yellowish blotches developed over the entire leaf surface.

In pepper definite calico symptoms developed, very like those found in potato. No indications of necrosis were evident (Fig. 6, A). Since the symptoms in this host differed so much from those produced by every one of the other yellow mosaics studied, which invariably produced a severe necrosis, generally resulting in death of the plant, it was considered of interest to determine whether or not calico-affected pepper plants would protect against infection of viruses belonging to this group. Accordingly, 4 series, each consisting of 10 calico-affected pepper plants, were inoculated with the virus of aucuba mosaic, Canada streak, tuber blotch, and pseudo-net necrosis,

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[2] Unpublished data furnished by C. F. Clark, Bureau of Plant Industry, U. S. Department of Agriculture.

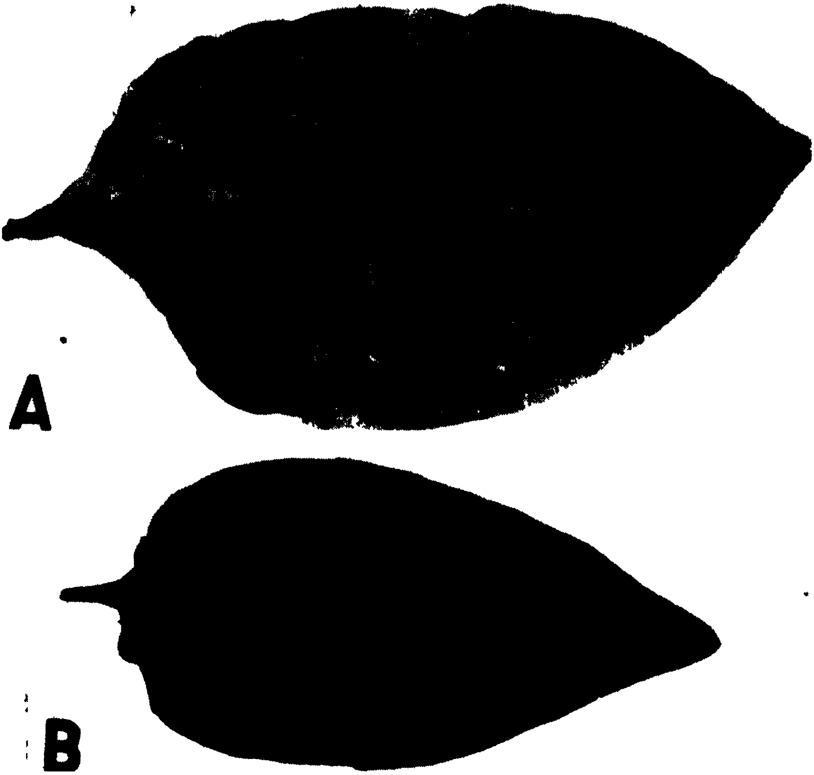

FIG. 6. A. Leaf from pepper plant (*Capsicum annuum*) infected with the calico virus free from virus X. B. Leaf from healthy control plant.

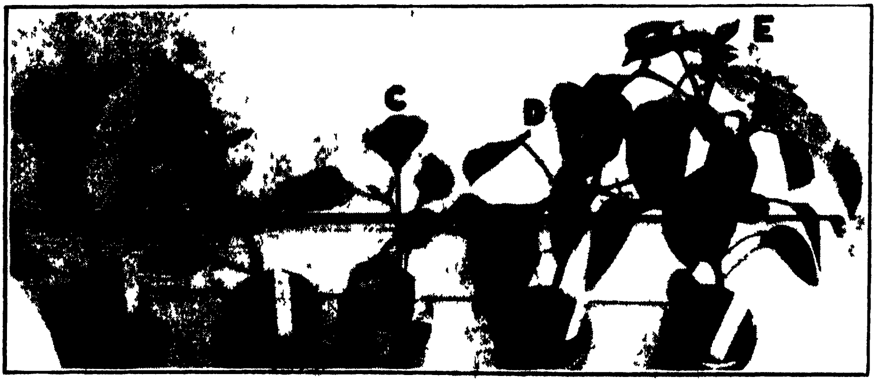

FIG. 7. Pepper plants infected with calico were inoculated with viruses belonging to the aucuba-mosaic group, to determine whether the presence of the calico virus in pepper affords protection against infection of the aucuba-mosaic viruses. The following plants are representative samples from the different series that were inoculated: A. Tuber blotch; B. Pseudo net necrosis; C. Aucuba mosaic; D. Canada streak; E. Calico-infected plant not inoculated. The fact that all inoculated plants became infected with the respective viruses shows that calico did not provide protection against infection of the viruses of the aucuba-mosaic group.

respectively (Fig. 7). Within 2 weeks necrotic lesions on the leaves and streaking of the petioles developed in every inoculated plant. This demonstrated definitely that calico did not protect against infection by any of these viruses. These results would indicate the absence of any natural relationship between calico and the other yellow mosaics, if failure to provide protection be a reliable criterion.

The calico virus is very sensitive to external conditions and is easily destroyed. It failed to pass through either an L7 or L5 candle filter. A very small percentage of infection is obtained if the inoculum is diluted more than 1–10. The thermal inactivation point is considerably lower than that of the viruses belonging to the aucuba mosaic group (Table 2).

TABLE 2.—*Properties of calico virus on Nicotiana sylvestris as host*

Thermal inactivation point	Number infected[a]	Tolerance to dilution	Number infected[a]	Longevity *in vitro* at 15°C.	Number infected[a]
°C.				*hours*	
40	15/2	1–10	20/14	20	15/6
42	15/0	1–100	20/3	28	15/2
45	15/0	1–200	20/0	44	15/1
48	15/0	1–300	20/1	Control	15/10
50	15/0	1–500	20/0		
52	15/0				
54	15/0				
56	15/0				
58	15/0				
60	15/0				
63	15/0				
Untreated	15/6				

[a] Numerator, number of plants inoculated. Denominator, number of plants infected.

DISCUSSION

Clinch *et al.* (4), in their grouping of potato viruses, propose to establish a new group, namely, viruses of the F type, in which should be included pseudo-net necrosis, tuber blotch, and aucuba mosaic. On the basis of performance and property studies, they have come to the conclusion that tuber-blotch virus and that of pseudo-net necrosis are probably identical, and that the aucuba virus is a related but distinct form.

The experiments reported in this paper confirm the results secured by Clinch *et al.* (4). The writer considers tuber blotch and pseudo-net necrosis to be synonyms of the same disease.

Canada streak, a newly described disease, appears to be induced by a single virus, rather than a virus complex, and should, therefore, be referred to as a strain of aucuba mosaic that produces necrosis. On the basis of property studies and serological reactions, aucuba mosaic and Canada streak seem to be more closely related to each other than is pseudo-net necrosis or tuber blotch to either one of them.

Although calico virus produces a yellow type of mottling on potato varieties, somewhat similar to that caused by aucuba-mosaic virus, it must be concluded, on the basis of symptoms on other solanaceous plants, physical properties, and protective inoculations, that no natural relationship exists between the calico virus and those viruses of the aucuba mosaic group.

There is no evidence that either Canada streak or tuber blotch is found on potatoes in commercial fields in the United States; but, since the tuber-blotch symptoms may easily be confused with the nonparasitic internal brown spot, and foliar symptoms are often masked under field conditions, it is advisable to keep this potentially serious disease in mind when diagnosing internal necrotic spots in potato tubers.

SUMMARY

Pseudo net necrosis and tuber blotch were found to be identical. Canada streak, a newly described disease, causes necrotic blotches in tubers of all the potato varieties tested and is considered a distinct strain of aucuba mosaic.

The aucuba mosaic group of viruses includes aucuba mosaic, pseudo-net necrosis or tuber blotch, and Canada streak.

No natural relationship was found between the viruses of the aucuba mosaic group and the calico virus. There is no evidence that either tuber blotch or the Canada streak virus is found in the United States.

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RELATION OF MOISTURE TO INFECTION WITH SOME DOWNY MILDEWS AND RUSTS[1]

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INTRODUCTION

The extensive literature on the relation of moisture and other environmental factors to plant diseases caused by downy mildews and rusts has been reviewed by Foister (2, 3) and will be referred to only briefly here. The downy mildews as a group are generally considered to be especially favored by wet weather under natural conditions. Free water usually is considered essential for sporangial germination whether germination be by means of a germ tube or by swarmspores. For infection with the urediospores of rust fungi the necessity of wet weather does not appear so well established as for downy mildews, and there is more disagreement between investigators. The prevalent opinion seems to be that free moisture is necessary for urediospore germination and subsequent infection. The general practice is to provide free moisture on the leaves in rust inoculations, though reports of comparative inoculation trials in a humid atmosphere with and without free water added to the leaves have not been found by the writer.

Downy mildew of hop, caused by an organism characterized by sporangial germination only by swarmspores, has, since 1934, been serious in Sonoma County, California, only in seasons characterized by frequent rains during the early growing season. On the other hand, downy mildew of onions caused by an organism known to show sporangial germination only by germ tubes, has been serious in California in seasons of very low rainfall, though of abundant dew, even in the same locality where hops, with an abundance of downy mildew inoculum present, showed little spread of the disease. The abundance and seriousness of other downy mildews and of several rusts in regions and seasons characterized by little or no rain during part at least of the epidemic season, seemed to justify further study of the moisture relations of certain members of these important groups of plant pathogens.

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[1] The assistance of non-technical employees of the Federal Works Progress Administration is acknowledged.

This experimental study is confined principally to attempts to determine whether or not the addition of free mosture to plants is desirable in inoculations with downy mildews and rusts. The relation of leaf temperature to condensation of moisture on leaves also is treated.

MATERIALS AND METHODS

To study with accuracy the moisture conditions for germination and infection with parasitic fungi requires a much higher degree of temperature control than was used in this study. In a chamber in which the space is saturated with water vapor a very small lowering of the temperature of objects within the chamber, such as the leaves of the experimental plants, may cause deposition of moisture and may, consequently, importantly affect the results.

Although the methods used in this study are considered similar to those used by other investigators in closely related studies, the hitherto unpublished results here recorded have such an important bearing on inoculation methods as to seem to justify reporting them at this time.

Inoculation studies were made with the following diseases: onion downy mildew (*Peronospora destrucor* (Berk.) Caspary on *Allium cepa* L.), spinach downy mildew (*Peronospora effusa* (Grev.) Ces on *Spinacea oleracea* L.), hop downy mildew (*Pseudoperonospora humuli* (Miy. and Tak.) Wilson on *Humulus lupulus* L.), cucumber downy mildew (*Pseudoperonospora cubensis* (Berk. and Curt.) Rostew on *Cucumis sativus* L.), clover rust (*Uromyces fallens* (Desm.) Kern. on *Trifolium pratense* L.), bean rust (*Uromyces phaseoli* (Pers.) Wint. on *Phaseolus vulgaris* L.), snapdragon rust (*Puccinia antirrhini* Diet. and Holw. on *Antirrhinum majus* L.), and sunflower rust (*Puccinia helianthi* Schw. on *Helianthus annuus* L.). Each of these fungi penetrates its host through the stomata.

To determine whether or not free moisture was necessary for spore germination, tests were performed on glass slides with the sporangia of onion mildew, and the urediospores of the rusts of bean and sunflower at 16° and 19° C. Spores were dusted onto glass slides. One half of each slide was atomized with water; the other half was not. The slide, with dusted surface oriented up, was supported on glass rods in the cover of a Petri dish containing 10 cc. of water. After the open dish was allowed to remain at the test temperature for about 5 minutes the base of the dish was inverted over the slide of spores in the Petri dish cover, thus giving a water-sealed germination chamber. No water condensed on the unatomized portion of the slide under these conditions.

With the downy mildews of spinach and hop the inoculum consisted of sporangia collected in the field. The sporangia of the other downy mildews and the urediospores of all the rusts studied were grown for inoculation purposes on greenhouse plants and were used within a few days of sporulation, unless otherwise mentioned. Healthy plants for inoculation purposes were grown in 3-, 4-, or 5-inch pots in the greenhouse and were generally used within a month of seeding or transplanting, except for hop mildew, for which detached leaves of field plants were employed. In all cases the experimental plants or leaves were carefully selected for comparability at the beginning of a test. Inoculations were performed by dusting the dry[2] host leaves with the dry spores. Generally, the plants or leaves were held in an inverted position in a garbage can and all plants or leaves in a given test group were dusted simultaneously in the same can and as uniformly as possible. Applications of spores to onions were made with the plants held in a horizontal position.

Inoculated plants were incubated in closed garbage cans, each containing about an inch of water. When soil saturation was desired, as was generally the case, the pots were set in about an inch of water in the garbage can. When the soil moisture was held in a reduced state the pots were set on a rack above the surface of the water. The garbage cans were held in a dark room, where the temperature remained within ½ a degree of 14° C. during these tests. Inoculations generally were made in the afternoon and the inoculated plants were allowed to stand in the darkroom for about 10 minutes to reach the temperature of the room and then placed overnight in the garbage-can moist chambers. The group of inoculated plants to which free water was added was atomized with water at 14° C. immediately before placing the plants in the moist chamber. The incubation period was generally about 15 hours, at the end of which the plants were returned to the greenhouse. In some tests there were check plants dusted with rust or downy-mildew spores and held continuously on the greenhouse bench. Such plants did not become infected.

Detached leaves were inoculated with rust by dusting the spores onto the lower (dorsal) surface of the test leaves, and incubating these leaves on the surface of dry, paraffined wire screens in closed Petri-dish moist chambers containing about 10 cc. of water. The inoculated leaves were incubated with their upper or lower surfaces down on the screen and toward the water as desired.

Soil moisture determinations were made from samples taken immediately after the plants were removed from the incubation chambers. Samples of 10 to 30 grams each were weighed immediately and after 2 days at 90° C. The soil in the pots standing in water was assumed to be at saturation, and with the greenhouse soil used contained about 30 per cent of the dry weight of the soil as water. The degree of saturation of the dry soil samples was calculated from this. Plants subjected to reduced soil moisture before and during incubation were returned to a normal watering schedule after removal from the incubation chambers.

To determine the effect of turgidity of spores on infection, spores were dried by holding wilted, detached, spore-bearing leaves in a dry room at about 22° C. for the period stated. In each test the dried and undried spores were examined microscopically in paraffin oil and the dried spores were observed to be much more collapsed than the undried spores.

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[2] Dry is used here to mean that no free moisture was evident.

MEASUREMENT OF LEAF TEMPERATURES

Measurements of onion-leaf temperatures were made by means of ordinary laboratory-type mercury thermometers, calibrated to an estimated 0.1° C. and inserted into the hollow interior of onion leaves held in a vertical or slightly inclined position. Leaves of a suitable diameter were chosen, the tips were cut off, and the thermometers were inserted so that the mercury reservoir was about 3–4 inches within the leaves and fitted snugly to them. The mercury bulbs of the leaf thermometers were usually about a foot above the soil surface level and the air thermometers were held at the same level. The air thermometers and test leaves were not protected from rain or radiation.

During the course of this study a total of 2857 readings of air and leaf temperatures have been made within a period of 73 days. The readings were obtained under a variety of outdoor weather conditions, and in the greenhouse and darkrooms. About half of the readings were obtained at night partly to determine the conditions during dew formation on onion leaves. Most of the night readings, however, were made before dew deposition began. The average deviation from the mean for the period of 100 readings of leaf temperature outdoors, at night (42 periods of observation), was 0.13 ± 0 012° C. (standard error). The average difference between duplicate readings on different leaves was approximately twice this value, and the maximum observed difference between duplicate thermometers in leaves outdoors at night in a comparable environment was 0 7° C. The variability of air-temperature readings at night was generally less than that of leaf temperatures.

EXPERIMENTAL RESULTS

Effect of Free Moisture on Spore Germination

Sporangia of onion mildew and urediospores of bean and sunflower rusts failed to germinate when seeded onto dry slides in Petri-dish moist chambers; while similarly seeded spores which were later atomized with water, germinated well. When spores were seeded dry onto the inner surface of the cover of a Petri dish and suspended over the water-containing half, there was frequently sufficient condensation of moisture on the cover to induce germination. The writer believes that free moisture is necessary to the germination of sporangia and urediospores thus studied on glass slides.

Effect on Infection of Adding Free Moisture to Inoculated Leaves

Results of tests to determine the effect of free moisture on infection with several downy mildews and rusts are given in table 1. With bean rust, snapdragon rust, and cucumber mildew, several more tests were performed than are here reported, but the tabulated results are typical. With the downy

TABLE 1.—*Effect of free moisture on infection with some downy mildews and rusts*

Disease and date of test	Units compared	Criterion of infection	Amount of infection	
			"Dry" leaves	Atomized leaves
Onion downy mildew				
May 1, 1938	Pots of seedling plants	Percentage of plants sporulating	45 per cent (42)[a]	67 per cent (43)
Aug. 5, 1938	"	"	33 per cent (9)	17 per cent (18)
Aug. 12, 1938	"	"	77 per cent (47)	56 per cent (50)
Aug. 16, 1938	"	"	87 per cent (112)	84 per cent (81)
Spinach downy mildew				
May 2, 1938	Pots of seedling plants	Percentage of plants sporulating	64 per cent (42)	69 per cent (52)
May 3, 1938	"	"	73 per cent (26)	85 per cent (34)
Hop downy mildew				
April 24, 1938	Detached leaves	Total lesions on 4 leaves	8	336
April 28, 1938	"	Total lesions on 3 leaves	0	1200
May 5, 1938	"	Total lesions on 4 leaves	0	325
Cucumber downy mildew				
June 28, 1938	Potted plants	Total lesions on 4 plants	0	44
July 9, 1938	"	"	3	681
Aug. 12, 1938	"	"	26	170
Aug. 17, 1938	"	"	415	1976
Clover rust				
Dec. 12, 1931	Detached leaflets	Total pustules on 1 leaflet	324	582
Bean rust				
July 9, 1938	Potted plants	Total pustules on 6 cm^2	131	266
Aug. 4, 1938	"	Total pustules on 4 cm^2	353	183
Aug. 5, 1938	"	Total pustules on 4 leaves	970	2062
Sept. 5, 1938	"	Total pustules on 8 in^2	1315	1050
Snapdragon rust				
Nov. 6, 1936	Detached leaves	Total pustules on 20 leaves	496	637
July 29, 1938	Potted plants	Total pustules on 5 cm^2	246	354
Aug. 12, 1938	Potted plants	Total pustules on 8 leaves	428	626
Aug. 13, 1938	"	Total pustules on 4 leaves	211	53
Sunflower rust				
Aug. 12, 1938	Potted plants	Total pustules on 10 leaves	576	516
Aug. 13, 1938	"	Total pustules on 4 leaves	131	274
Sept. 5, 1938	"	Total pustules on 8 in^2	882	405

[a] Number in parenthesis is number of plants on which percentage was based.

mildews of the onion and spinach and with all the rusts tested, there was no significant and consistent difference in the amount of infection on the atomized and nonatomized plants. Although the inoculated leaves were dry when placed in the incubation chamber, careful observation revealed that they were generally wet with condensed moisture when removed about 15 hours later. Only in the case of the detached hop leaves and clover leaflets was no moisture ever observed on the inoculated leaflets, though the amount of moisture on the nonatomized leaves of the other plants was sometimes inconspicuous or nonapparent. Always, there was much less than on the atomized leaves. That this was the moisture of condensation, rather than that of guttation, was indicated by its uniform distribution and, specifically, by some tests with beans. It is known that guttation is caused by root pressure, while condensation is wholly independent of root pressure. Therefore, detached bean plants with their stems in flasks of water were compared with beans growing in soil. The occurrence of a film of fine drops of moisture and the amount of subsequent rust infection of inoculated leaves was similar for the detached and rooted plants. True guttation moisture was observed on onions and cucumbers, but is not believed to have affected the general results.

With downy mildew of the hop, a small amount of infection occurred on the unatomized leaves in 1 out of 3 tests, though the total infection on the unatomized leaves was only about 0.4 per cent of that on the atomized leaves. With cucumber downy mildew, infection occurred on the unatomized leaves in 3 out of 4 tests, and the total infection on the unatomized leaves was about 15 per cent of that on the atomized leaves. With downy mildews of hop, cucumber, and onion there is an interesting correlation between the method of sporangial germination and the amount of infection on unatomized leaves. So far as the writer is aware, the sporangia of the first, *Pseudoperonospora humuli,* germinate only by swarmspores. Germination presumably would occur only in the presence of an abundance of free water. In agreement with this presumption there was only a trace of infection on the unatomized leaves. The germination of the sporangia of *Pseudoperonospora cubensis* (cucumber mildew) may be by germ tubes or by swarmspores. More generally it is by the latter, and might be expected to be more copious in the absence of an abundance of free moisture than would be true of hop mildew. Accordingly, considerably more infection occurred on the unatomized cucumber leaves than in the case of hop mildew. With *Peronospora destructor,* the cause of onion mildew, sporangial germination occurs only by germ tubes, so far as the writer is aware. Therefore, a still greater tolerance to a decrease of free moisture might be expected. In the tests reported the infection with onion mildew was as great on unatomized as atomized leaves, and much greater, relatively, than for hop or cucumber mildew under similar conditions. The writer, however, does not feel that such a correlation and inferred generalization is entirely safe, for the host plants concerned may show as much individuality as the fungi involved. With detached hop leaves, for instance, no condensation of moisture on the lower side of the leaves under these experimental conditions was ever observed, while condensation of moisture on the sides of onion leaves was frequently observed.

The luxuriant infection secured in darkness with these fungi, which penetrate through the stomata, indicates that they, as do many rusts (5), require neither light nor open stomata for infection, though no observations on stomatal openings were made in this study.

Effect of Soil Moisture on Infection

Results of all tests performed to determine if soil moisture at the time of inoculation had any effect on the success of inoculations are given in table 2.

TABLE 2.—*Effect of soil moisture on infection with some downy mildews and rusts. 1938*

Disease	Date of test	Soil moisture of dry soil percentage of saturation	Amount of infection[a]			
			Plants in dry soil		Plants in saturated soil	
			Dry leaves	Atomized leaves	Dry leaves	Atomized leaves
Onion mildew	Aug. 6	49	54 per cent (11)		33 per cent (9)	17 per cent (12)
Cucumber mildew	" 6	51	0		7	28
" "	" 12		11	38	15	132
Bean rust	July 10	69	6		100	197
" "	" 25		0		207	506
" "	" 29		178		66	90
" "	Aug. 6	56	146		970	2062
" "	" 9	39	520		395	
Snapdragon rust	July 29		269		246	354
" "	Aug. 6	34	157		49	118
" "	" 13		72	109	211	53

[a] For onion mildew the amount of infection is recorded as the percentage of plants infected of the total inoculated. The number inoculated is given in parentheses. For the other diseases the amount of infection is recorded as the number of lesions on comparable areas of leaf tissue for each test.

With such extreme variability of results no reliable conclusions can be drawn as to the quantitative effect of free moisture on the leaves for plants in a dry or wet soil, but it appears that, with low soil moisture (34 to 69 per cent of saturation), there is a greater likelihood that dry (unatomized leaves) inoculations will give lower infection than obtains in a saturated soil. Low soil moisture, however, apparently prevented infection in only two cases.

Effect of Turgidity of Spores on Infection

Results of all tests to determine the effect of turgidity of spores on resulting infection are given in table 3. Only in the one test with snapdragon rust was there an apparent marked reduction in infection, due to the use of

TABLE 3.—*Effect of turgidity of spores on infection with some downy mildews and rusts. 1938*

Disease	Date of test	Period of drying of spores	Amount of infection[a]			
			Dried spores		Fresh spores	
			Dry leaves	Atomized leaves	Dry leaves	Atomized leaves
Onion mildew	Aug. 12	8 hrs.	84 per cent (19)	81 per cent (26)	71 per cent (28)	29 per cent (24)
Onion mildew	" 16	7 hrs.	90 per cent (40)	78 per cent (23)	85 per cent (33)	80 per cent (20)
Bean rust	" 8	11 days	213	203	372	775
Snapdragon rust	" 12	10 days	1	721	428	628

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[a] For onion mildew the amount of infection is recorded as the percentage of plants infected of the total inoculated. The number inoculated is given in parentheses. For the other diseases the amount of infection is recorded as the number of lesions on comparable areas of leaf tissue for each test.

the dried spores on unatomized leaves. The period of drying in the other tests, however, may not have been properly chosen to show adequately the effect of drying of spores on the amount of infection. From this evidence it cannot be safely concluded that there was any marked difference in dried and fresh spores. Nevertheless, the experiments of Napper (6) are of interest here. She found that the sporangia of *Cystopus candidus* did not germinate unless first dried so that their maximum content of water had been reduced by about 30 per cent.

Leaf Temperatures

Numerous measurements indicate that except in exposure to sun, onion leaf temperature is lower than that of the surrounding air. When exposed to the sun the leaves of the onion show a temperature usually higher than that of either shaded or unshaded situations. During rainfall the leaf temperature is about equal to or slightly exceeds that of the surrounding air. Of all paired measurements of air and leaf temperature outdoors at night, with readings at about 1- to 2-hour intervals from dusk to 11 P.M., and with air temperatures varying from 1.7 to 17.4° C., onion leaves were 0.26 ± 0.044° C. cooler than the air in clear, still weather (44 observations); 0.75 ± 0.071° C. cooler than air during clear, windy weather (20 observation); 0.34 ± 0.084° C. cooler than air in cloudy weather (17 observations); and 0.18 ± 0.05° C. higher than air during rain (4 observations). In dark, constant-temperature (14° C.) moist chambers, onion leaves were 0.23 ± 0.07° C. cooler than the surrounding air (23 observations). The marked increase in leaf temperature, compared to air temperature when reradiation to the sky was reduced as recorded by Curtis (1), was not observed in this study. This may be due in part to the writer's attempt to measure the temperature of the leaf interior and not that of the surface, and also in part to the fact that the leaves were held in an approximately vertical position during temperature measurement.

In table 4 are recorded the atmospheric and leaf conditions observed at the first appearance of dew on onion leaves for several nights outdoors. The striking features of these records are the low measured relative humidities at which dew condensed on onion leaves (average 84, minimum 68), the marked temperature inversion near the ground level, and the low leaf temperatures as calculated from the air temperature at the leaf level and the relative humidity from the sling psychrometer. Possibly the calculated leaf temperatures were lower than the measured ones partly because the temperature at the leaf surface, where condensation occurred but was not measured, was lower than the measured temperature in the interior of the leaf. A more important cause of this discrepancy probably is the fact that the relative humidity at the leaf surface is higher than at 4 feet above the soil level, where it was measured (4). This higher humidity at the leaf surface could be caused by the generally lower temperature of the air near the soil level, by the lower temperature of the air adjacent to the colder leaf,

TABLE 4.—*Atmospheric and leaf conditions during dew formation on onions*

Time	Sky	Air temperature		Relative humidity from sling psychrometer	Leaf temperature as plus or minus air temperature	
		Still thermometer at leaf level (approx. 1 ft. above soil level)	Dry bulb of sling psychrometer (approx. 4 ft. above soil level)		Observed with thermometers at leaf level	Calculated from air temperature at leaf level and depression of wet bulb of sling psychrometer
		°C	*°C*	*Per cent*	*°C*	*°C*
7 PM Dec. 16, 1938	light clouds	9.65	9.88	91	−0.16	−1.59
7 PM Dec. 20, 1938	clear	7.30			−0.50	
6:45 PM Dec. 21, 1938	"	7.40	9.17	80	−0.68	−1.84
7 PM Dec. 26, 1938	"	7.00	8.50	69	−0.27	−4.22
7 PM Dec. 27, 1938	"	6.70	8.02	93	+0.10	−1.14
6 PM Dec. 28, 1938	"	7.40	8.70	88	−0.38	−0.73
6:30 PM Dec. 29, 1938	"	7.20	8.00	93	−0.22	−0.53
11:30 PM Dec. 30, 1938	"	3.80	5.18	93	+0.35	−1.08
10 PM Jan. 31, 1939	"	1.75	3.39	83	−0.70	−1.19
7:40 PM Feb. 1, 1939	"	2.95	4.28	68	−0.28	−4.06
8:10 PM Feb. 4, 1939	"	4.75	6.50	86	−0.05	−0.31
8:30 PM Feb. 6, 1939	"	3.90	5.45	83	−0.18	−1.12
9:30 PM Feb. 13, 1939	"	6.50	8.33	69	−0.75	−2.17
9:30 PM Feb. 14, 1939	light clouds	9.10	10.17	94	−0.30	−0.21
Average		6.10	7.35	84	−0.29	−1.55

and by the transpiration of the leaf. Observations with a stationary psychrometer, with which air was pulled horizontally by means of a fan over the wet and dry bulb thermometers, showed that the relative humidity near a soil or grass surface on cool, still nights was consistently higher than at 4 feet above such a surface.

If slight changes in air temperature alone were responsible for the condensation of moisture on leaves and the consequently favorable conditions for infection by dry spores applied to these leaves, then it should be possible to increase or decrease the expression of this phenomenon by manipulation of air temperature. Open Petri dishes of bean, snapdragon, and sunflower leaves, dusted with spores of their appropriate rust fungi, were placed at 13° and 19° C. and closed after 10 minutes. One dish of each series was left at 13°, one at 19°, another was transferred from 13 to 19° after 30 minutes, and still another from 19 to 13° after 30 minutes. After 21 hours the leaves were returned to the laboratory, dusted with sulphur to prevent any further infection, and the degrees of infection recorded after 12 days. If changes in air temperature were a major factor in bringing about condensation of moisture on the leaves and consequent infection, then infection should be highest on dishes of leaves transferred from 19 to 13° C., lowest for leaves transferred from 13 to 19° and intermediate for those at constant temperature. The results given in table 5 show no such tendency, however, and there is no significant difference apparent between the 4 types of treatment. Another test gave similar results.

TABLE 5.—*Effect of temperature manipulations and orientation of detached leaves on infection with some rusts*

Host	Orientation of inoculated surface during initial incubation	Temperature during initial incubation			
		13° C. continuous, number of pustules per leaf	19° C. continuous, number of pustules per leaf	13° C. for 30 min., then 19° C., number of pustules per leaf	19° C. for 30 min., then 13° C., number of pustules per leaf
Bean	up	9	0	0	0
	down	30	650	836	106
Snap dragon	up	31	34	25	13
	down	131	193	150	161
Sun-flower	up	38	5	32	46
	down	50	68	49	52

A consideration of the leaf surface on which moisture is condensed and its possible relation to source of moisture supply, radiation, and consequent infection, is pertinent to this study, though the writer's observations have been only superficial. With hops, the abundant condensation of moisture on the upper leaf surface and slight condensation on the lower surface during cool spring nights might be explained by the air being the principal source of moisture, and the leaves being cooler on their upper surfaces than on their lower because of radiation to the sky. Under these conditions there is very little infection of trained hops, presumably because little if any infection can take place through the upper surface (7), though an abundance of inoculum may be present.

With onions, relatively little moisture condenses on the normally erect leaves; on inclined or horizontal leaves, however, there may be heavy condensation on the upper surface, with very little on the lower. Under these conditions considerable spread of the disease may occur. Three factors that may account in part for the more abundant mildew on onions than on hops, under conditions of relatively clear spring weather, are the lower moisture requirement for onion mildew because of its direct germination, the dorsiventrality of hop leaves and its absence in onion leaves, and the fact that under ordinary culture conditions hop leaves are farther from the ground than are those of the onion.

ORIENTATION OF LEAF SURFACES

Orientation of leaves may have an important bearing on the incidence of infection on detached leaves in moist chambers. Besides the results with bean, snapdragon, and sunflower rust presented in table 5, a total of 15 tests of the effect of orientation of leaves on infection with snapdragon rust have been made in dish cultures. In all cases the infection was much greater when the dry, inoculated leaves were incubated with their dorsal (or occasionally ventral) inoculated surfaces facing downward, than when the inoculated surface faced upward during the initial moist chamber incubation period. In most of these tests the principal source of moisture was the free water in the base of the water-sealed Petri dish. When wet filter paper was applied to upper and lower parts of the dish or to the cover only, the difference in infection attributable to orientation of the leaves was much less. Infection was, however, still greater when the inoculated surface was oriented downward. These results might be considered as evidence of a negative geotropic tendency of the germ tubes of the urediospores of these rusts; but no such tendency could be observed when germination occurred on vertically placed glass slides or on agar. A more likely explanation is the greater tendency for moisture to condense on a surface facing downward than on one oriented upward, under ordinary incubator conditions, though this is opposite to observed field conditions. In the incubators the sources of heat were the basally placed electric heaters, which radiated heat from below and may have caused differences in the heating of objects within the incubator, while reradiation from the leaves presumably was slight. In these tests condensation on the leaves was not always observed, but, when observed, it was more abundant on the leaf surface oriented downward, tending to explain the greater infection occurring through this surface

SUMMARY

Inoculations of downy mildews of onion, spinach, hop, and cucumber and the rusts of clover, bean, snapdragon, and sunflower were successful when dry spores were added to dry leaves and the inoculated plants were incubated in moist chambers at constant temperature.

Only in the case of mildews of hop and cucumber, diseases caused by fungi with a swarmspore stage in the germination of their sporangia, was the infection markedly less, when no free water was added to the inoculated leaves, than when the inoculated leaves were atomized with water. In these tests macroscopically evident free moisture frequently condensed on the leaves of the unatomized plants.

Maintaining the inoculated plants in dry soil during the moist-chamber incubation period, or using dried spores as inoculum, had no marked effect on the above results, though these treatments did reduce the amount of infection on unatomized leaves in some cases.

More rust infection occurred on detached leaves of snapdragon, bean, and sunflower when the inoculated surface was faced downward than when faced upward during the incubation period.

Onion leaves, outdoors at night or in dark moist chambers, generally registered a lower temperature than that of the surrounding atmosphere. This lower leaf temperature is believed to be primarily responsible for the condensation of moisture on the leaves and the consequently favorable conditions for infection.

Because of the condensation of moisture on the leaves in these tests, it cannot be safely concluded from the results presented whether or not free moisture is necessary for infection with the fungi studied. The writer is of the opinion that in a humid atmosphere the temperature of the leaves, lower than that of the surrounding air, is usually sufficient to cause the deposition of enough moisture to induce spore germination and infection with the fungi studied. With the downy mildews of hop and cucumber a greater amount of free moisture is probably necessary than in the case of the other fungi studied.

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A TECHNIQUE FOR STUDYING HOST RESISTANCE AND PATHOGENICITY IN TOMATO FUSARIUM WILT

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INTRODUCTION

Studies of resistance of tomato (*Lycopersicon esculentum* Mill.) varieties, relatives, strains and breeding progenies, to the wilt caused by *Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and R., have been largely based on field trials. Such testing involves so much time, land, and expense that, at best, most investigators are able to study no more than a few thousand plants in a season, and it is impossible to make more than a single trial each year. Furthermore, unavoidable, large differences occur in the field in soil character, distribution of the parasite, climatic effects, presence of complicating diseases other than wilt, and numerous other factors affecting the tomato plant and the fungus. These variables make it almost if not wholly impossible to obtain results that agree very closely in replicate tests and have added to the difficulties in making accurate determination of the mode of inheritance of resistance to wilt. For the same reasons it has not been possible to make close comparisons of the virulence of different cultures of the tomato-wilt Fusarium in the field.

The method here described for determining resistance to Fusarium wilt of tomato was developed to meet the need of pathologists and geneticists for greater accuracy, dependability, speed, and lower cost of conducting tests. It has been found of value and used with marked success by the author in studying parasite-host relationships and the variations in pathogenicity among strains of the causal organism (9). Although there is still much to be desired, the technique represents a considerable improvement over former methods.

This technique depends on the control of several critical factors operative in infection by and subsequent progress of the wilt organism in the tomato plant. The procedure was based largely on the results of other workers who studied temperature relationships (1, 10), moisture requirements (2, 10), and effect of soil types (3, 4, 5, 8, 10), as well as the results of soil sterilization (3, 7). Any findings by workers other than the writer that have been utilized in the technique described here, have been carefully verified in the present studies.

Besides the usual laboratory facilities, the method requires a greenhouse where air temperatures may be fairly well maintained, night and day, around 27° C. (about 80° F.), and thermostatically controlled equipment for keeping reasonably constant soil temperatures. Wisconsin soil-temperature tanks and benches for soil equipped with electrically heated cables designed for use in greenhouse propagating beds both have been found satisfactory. The technique is described as developed and used in the laboratory and greenhouses at the U. S. Horticultural Station, Beltsville, Maryland.

PREPARATION OF SOIL

The soil consisted of a mixture of 2 parts of coarse sand to 1 of moderately fertile greenhouse potting soil. The soil was used repeatedly without addition of fertilizer, and was steam-sterilized for 4 hours at 10 pounds' pressure before each test. The soil usually was allowed to stand from 2 to 7 days, after sterilization, before planting; but, in some cases, seedlings were inoculated and planted in the soil the day after it was steamed, with no apparent ill effects.

GROWTH OF SEEDLINGS FOR TESTING

Tomato seeds were planted in flats of sterilized soil and the seedlings pricked off into thumb pots at the appearance of the unexpanded first true leaf. These pots were placed on wooden benches or in clean wooden flats without soil or sand. The most satisfactory test plants were those growing at a moderate rate and not too succulent at transplanting time. Very tender plants became easily infected with *Fusarium*, but were highly susceptible to mechanical damage during the inoculation process. Plants usually were inoculated when about 5 inches high and at about the 4-leaf stage. No seedlings were lost or failed to respond satisfactorily if they had been carefully handled and properly grown. A greenhouse temperature of about

27° C. (80° F.) was found to be the most suitable for producing seedlings for this work. The period from seed planting to final transplanting of the inoculated seedlings into the testing beds was usually about a month but varied from 25 to 40 days, depending on light conditions and temperature.

PREPARATION OF INOCULUM

Field soil naturally infested with the tomato wilt organism was first tried as a source of inoculum. Infection of plants was uneven and not dependable when such soil was used for the second time in greenhouse benches, the results thereby differing from those of certain other workers on other fusarioses; e.g. Walker and Smith (7) in studying the cabbage yellows. In addition to the use of wilt-infested field soil, sterilized soil was heavily inoculated by addition of the tomato Fusarium grown on oat kernels, on sand-cornmeal cultures, wheat chaff, cooked rice, liquid media, and on potato-dextrose agar. Soil inoculated by such means gave erratic results in the greenhouse the second time plants were set in it after inoculation, and produced very little disease on plants in the third trials. Inoculation by pouring fungus suspensions derived from liquid cultures over the soil or in holes at the base of plants gave fair results, but infection of plants took several weeks. In some cases as long as 3 months elapsed after inoculation before final wilt data could be recorded. Such soil was unsatisfactory when used again without adding more inoculum of the organism.

A method was next developed involving the dipping of the roots of the plants in a water suspension of *Fusarium* made from cultures grown in a liquid medium. A satisfactory culture solution was found to be the Tochinai (6) medium: peptone 10.00 g., monopotassium phosphate 0.50 g., magnesium sulphate 0.25 g., maltose 20.00 g., and water 1000.00 cc., autoclaved at 15 pounds' pressure 30 minutes. Flasks of 200 cc. capacity containing 100 cc. of the medium were used, and each was inoculated with a bit of agar culture of standard age, and about 1 cm. in diameter.

Cultures were shaken twice during the second day and were thereafter undisturbed. They were incubated at about 27° C., for 5 or 6 days. By this time a partly submerged, floating mat had been formed from which the excess liquid was decanted. This liquid was found to be quite toxic to tomato plants and so was not included in the suspension. The mat was transferred to a bowl, about 25 cc. of sterile water was added and the mat beaten for about 3 minutes with an electric egg beater, after which water was added to make a volume of 400 cc. Ten flasks of cultures produced enough inoculum to treat over 1000 seedlings.

INOCULATION AND TRANSPLANTING

At the time of inoculation, the tomato seedlings were knocked out of the thumb pots and the roots washed free of soil under running tap water. No particular care was taken to avoid root injury, indeed in some cases slight but not excessive root pruning was practiced. The washed roots were dipped in the inoculum and the seedlings planted, while dripping, into the test benches and watered. Excellent results were obtained from seedlings planted as close as 1½ in. in the row with 4½ in. between rows. Spacings were varied without appreciable effect.

The question arose as to the necessity of sterilizing the bottoms and sides of plant pans or benches used in the *Fusarium* studies. Galvanized iron pans in which plants had died following inoculation with virulent *Fusarium* cultures were variously treated as follows: steam-sterilized at 10 pounds' pressure for 6 hours; washed in a strong soap solution; treated with 10 per cent formaldehyde solution; flushed out with tap water and dried; and nontreated except for being scraped and brushed clean of adhering soil particles. Bench compartments in which virulent types of *Fusarium* had caused death of plants were also treated in several ways, as: cleaned out and the soil brushed off the sides in some cases, or washed off with water, or sterilized with 10 per cent formaldehyde. A layer of sand in which the heating cables were buried on the bottom of the benches was left undisturbed. Pans and benches were then filled with sterilized soil 6 to 8 in. deep and healthy noninoculated Bonny Best plants were planted in these and grown 12 days in exactly the same manner as during the inoculation tests. From over a thousand plants of this highly susceptible variety thus treated, not one was diseased. Thus it appeared that no infection of plants resulted from contaminated containers during the short test period if the plants were set in sterilized soil with the roots 2 to 4 in. away from the container walls and bottoms

TEMPERATURE AND MOISTURE CONDITIONS

Test benches or soil containers in temperature tanks were filled with 6 to 8 inches of the sterilized soil-sand mixture, and maintained by thermostatically controlled electric heating elements at temperatures of from about 25° to 28° C. (77° to 82° F.). Air temperatures were kept at approximately 24° to 30° C. (75° to 86° F.). Both soil and air temperatures are important factors influencing infection and severity of the tomato wilt disease and therefore need careful attention and control if comparable results are to be secured in repeated experiments.

Plants were watered carefully with ordinary tap water and the soil moisture kept within a range optimum for growth. It is known that soils excessively dry from the beginning check the growth of plants and delay wilt expression, and also wilting of the plants is apparently inhibited almost indefinitely when they are grown in soils near the saturation point.

TIME REQUIRED

Under some conditions all seedlings of a uniformly susceptible tomato selection, when inoculated with a highly virulent Fusarium strain, have been severely affected by the disease at the end of 4 days in the testing bed. However in some instances, depending upon resistance of the plant material tested and the virulence of the culture used, plants have required from 2 to 3 weeks to show definite wilting of the leaves. By the use of a sufficiently virulent culture under the conditions above described, 6 to 10 days was ordinarily sufficient to secure adequate comparative resistance tests (fig. 1), or evaluations on progress of disease and relative pathogenicity of cultures of the fungus. The whole period necessary for a single test crop, from date of seeding to pulling plants and final note taking, is from 5 to 7 weeks. Successive lots of seeds for consecutive tests can be planted at 2-week intervals to have a new supply of plants in readiness as soon as each run is finished.

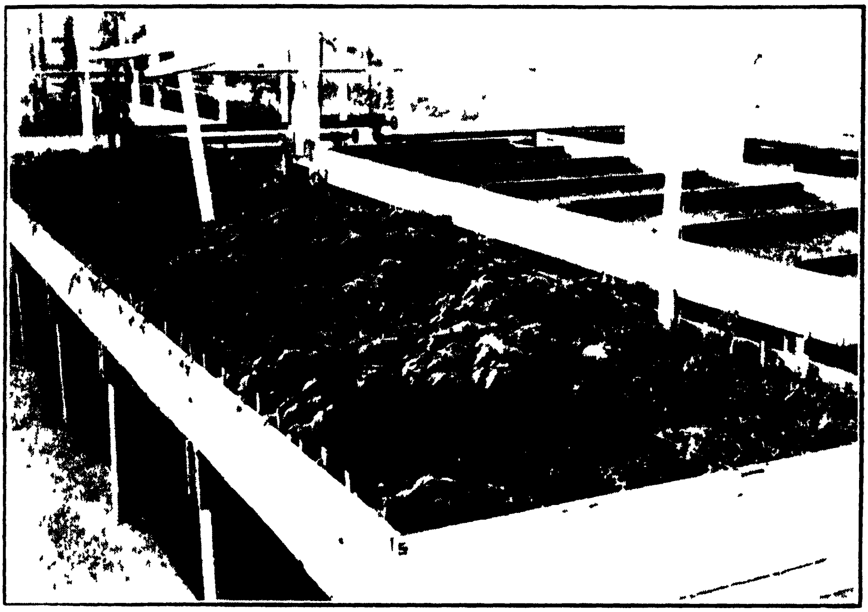

FIG. 1. Tomato plants of various strains being tested for resistance to Fusarium wilt by means of the technique described in this paper. Note marked differences in appearance of plant strains. In some all were severely wilted, in others all seemed healthy, in certain strains there were both wilted and apparently healthy plants. Note, on the right, benches of soil recently prepared with compartments for testing differences in pathogenicity of certain cultures of the tomato wilt Fusarium. (Photographed by W. S. Porte.)

DETERMINATION OF RESISTANCE TO WILT[1]

Through use of the technique described above, it has been possible to repeat a number of times experiments that gave comparable data demonstrating relative resistance to Fusarium wilt in tomatoes. The 3 tomato varieties discussed hereinafter were known to have different reactions under field conditions. The susceptible variety (Bonny Best) always has proved easily and severely diseased when infected. The resistant variety (Red Currant, *Lycopersicon pimpinellifolium* Mill.) has retained perfect resistance in the field under severe conditions that result in killing of all adjacent, fully susceptible plants. The partly resistant or intermediate variety (Marglobe) shows a capacity for outgrowing Fusarium infection under moderate conditions for disease production. Under unusually severe field conditions all plants of this intermediate, tolerant variety may finally succumb to the disease, but, compared with the susceptible variety, it takes a definitely longer time for complete wilting of infected plants.

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[1] The writer here wishes to acknowledge the aid of W. S. Porte who furnished seeds for these studies and supplied the field data presented in table 1.

Table 1 presents results on resistance trials by the use of the greenhouse technique in comparison with field trials. It will be noted that plants of the susceptible variety were equally susceptible in the greenhouse and the field. Plants of the resistant variety did not wilt either in the greenhouse or in the field. Plants of the variety intermediate in resistance showed some erratic behavior both in the field and in the greenhouse. In both the field and the greenhouse tests, plants of intermediate resistance required much longer to wilt than did susceptible plants. It is believed that the difference in wilting between the first three and the succeeding tests of Marglobe in the greenhouse was due mainly to the lower air temperatures that prevailed at first. Note, however, that there was comparatively little difference with regard to rapidity of wilting in Bonny Best.

NUMERICAL EVALUATION OF DISEASE SEVERITY

All macroscopically observable disease effects were considered in making the numerical evaluations. These effects included the external symptoms exhibited by the aerial part of the seedlings and also the discoloration of vascular elements. At the end of a predetermined growth period after inoculation, the plants were examined for disease symptoms, pulled, and

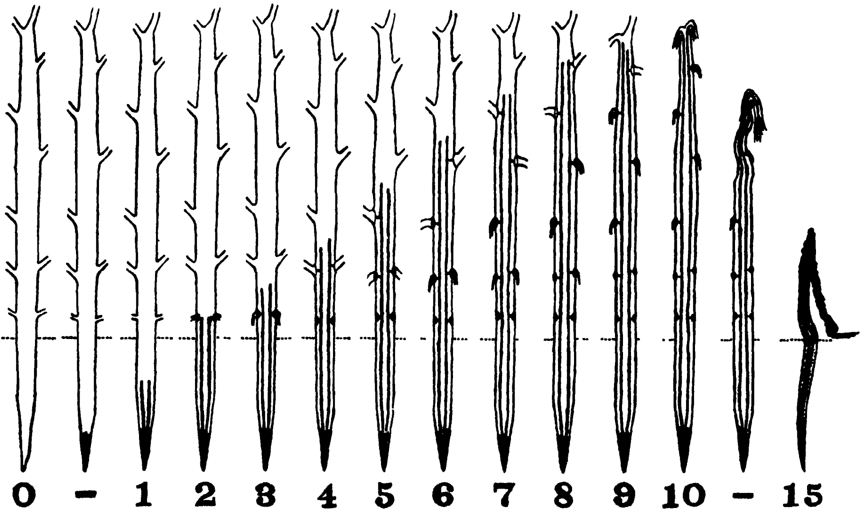

FIG. 2. Diagrams of tomato seedlings representing different disease evaluations based on macroscopically observed symptoms of Fusarium wilt: 0, no symptom; 1, infection evidenced by browned vascular bundles in true root, no leaf symptoms; 2, infection advanced to cotyledonary plate, chlorosis of cotyledons; 3, infection above cotyledonary plate; 4, infection to and slightly above first true leaves; 5, infection further advanced, first leaves chlorotic and wilting; 6, infection above middle part of plant, several basal leaves involved; 7, infection in over three quarters of length of stem, older leaves chlorotic; 8, infection within a few nodes of tip, leaves of all ages affected except some at top; 9, infection to within a short distance of tip bud, chlorosis of tip leaves, wilting of leaves below; 10, infection through whole plant, wilting of all leaves, stem still turgid and upright; 15, collapse and death after wilting of all leaves, breakdown of stem.

TABLE 1.—*Results of tests of three tomato varieties for resistance to the Fusarium wilt organism, in the field and with the greenhouse technique, using virulent forms of the wilt organism*

Variety	Date	Method of test	Number of plants		Period of time after inoculation		
			Planted	Completely wilted	First plant wilting	Last plant wilting	Total period
					Days	*Days*	
Susceptible (Bonny Best)	Summer 1936	Field	100	100			Growing season
"	" 1937	"	100	100			" "
"	" 1938	"	100	100			" "
"	Nov. 1937	Greenhouse	30	30	4	7	7 days
"	Dec. 1937	"	13	13	4	14	14 "
"	Jan. 1938	"	26	26	6	7	7 "
"	Aug. 1938	"	24	24	4	7	7 "
"	Sept. 1938	"	32	31[a]	5	9	18 "
"	Oct. 1938	"	45	45	5	7	7 "
"	March 1939	"	20	20	5	7	7 "
"	April & May 1939	"	20	20	5	9	9 "
Resistant (Red Currant)	Summer 1936	Field	100	0			Growing season
"	" 1937	"	100	0			" "
"	" 1938	"	100	0			" "
"	Nov. 1937	Greenhouse	30	0			32 days
"	Dec. 1937	"	13	0			24 "
"	Jan. 1938	"	32	0			41 "
"	Aug. 1938	"	20	0			7 "
"	Sept. 1938	"	31	0			18 "
"	Oct. 1938	"	20	0			16 "
Intermediate (Marglobe)	Summer 1936	Field	40	31[b]			Growing season
"	" 1937	"	20	20[b]			" "
"	" 1938	"	30	23[b]			" "
"	Nov. 1937	Greenhouse	30	30	15	32	32 days
"	Dec. 1937	"	14	14	10	24	24 "
"	Jan. 1938	"	28	28	17	28	28 "
"	Aug. 1938	"	16	14	8	18	18 "
"	Sept. 1938	"	8	6	8	14	14 "
"	Oct. 1938	"	50	50	9	16	16 "
"	Mar. 1939	"	20	18	9	18	18 "
"	May 1939	"	20	16	10	20	20 "

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[a] One plant found completely disease-free. Foliage type distinctly different from the susceptible strain, probably a chance seed mixture.

[b] Although these tolerant plants were killed by wilt, they survived much longer and bore more fruit than Bonny Best.

the stems and taproots split and cortex scraped away from the vascular strands to determine the extent of their discoloration. The relative degree of invasion of each plant was recorded on the numerical basis described below. With regard to internal effects it was sufficient in most cases to determine with the naked eye the extent of vascular discoloration, although, in critical cases a hand lens was used. After a little experience, two people, one examining plants and making evaluations and the other recording them, can easily obtain data on a thousand plants in a day.

Figure 2 illustrates the numerical values representing disease severity in tomato seedlings affected with Fusarium wilt, which may be described as follows: *No apparent disease,* value 0[2], no disease effects above ground and no observable discolored bundles in main taproot. *Primary infection,* value 1; no disease appearance above ground, with discolored vascular elements only in the true root region. *Very mild disease,* value 2; exterior symptoms usually shown only on cotyledons, with discolored vascular elements in the transition region and extending to the cotyledonary plate. *Mild disease,* value 3; the discolored vascular elements extend above the cotyledonary plate. *Medium disease,* values 4 and 5; some of the oldest leaves affected, discoloration of vascular bundles farther advanced up the base of the stem. *Severe disease,* values 6 and 7; some leaves of old and medium age affected, but the younger foliage and tip portion apparently healthy, discolored vascular elements extend through the middle sector of the stem. *Very severe disease,* value 8; condition in which some leaves of all ages except the youngest two or three at the tip are affected with darkened veins within a few nodes of the top. *Wilting severe,* value 9; first signs of wilting, such as yellowing, in the tip leaves with the discoloration of the vascular system extending along the whole length of the stem up to within a short distance of the tip bud. *Complete wilt,* value 10; all leaves, including those at the tip of the plant show wilt symptoms; stem still turgid and upright, and the darkening of the vascular system notable throughout. *Early wilt and collapse,* value 15; with severe general systemic necrosis following complete wilting, which had occurred two or more days before observation of the plant. There are, of course, points between 10 and 15 that may be interpolated, dependent upon the exact time of death of the plant. To determine this required individual marking of plants and notetaking twice a day until the final data were recorded.

In this numerical evaluation system a range of two numbers is used to express relative increments of disease severity under the "medium-disease" and "severe-disease" categories. The accuracy of assigning these values depends to a certain extent upon familiarity with expressions of the disease and, of course, care in observation. However, the numerical classifications are not difficult to use after some experience. As a result of extensive trial it was found that the numbers were quite dependable for recording disease stages. It is probable that in statistical analyses of plant disease evaluation data on small to medium populations, it may be necessary to group the numerical evaluations for best results. For example, the sixteen values may be grouped into 4 classes, as follows: 0, 1 and 2 into class 1; 3, 4 and 5 into class 2; 6, 7 and 8 into class 3; and 9 to 15 into class 4.

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[2] Isolation studies of several hundred root systems of inoculated plants with an evaluation of 0, have always shown that the roots were infected, although no observable discoloration was noted in the vascular elements.

Numerical Evaluations in Relation to Subsequent Disease Progress

It is well to note at this point that the plant-disease evaluations, described above (Fig. 1 and Table 2) were specially developed to use with seedling plants handled and grown for a definite period under the controlled conditions specified in this paper. The terms "mild," "medium," "serious" and "severe" are commonly used also in the field for evaluation of disease effects on older plants. It was interesting to see what later changes might occur in seedlings classed according to different disease values at 7 days after inoculation. Histories of many individual plants were followed for several weeks to observe what changes had occurred after successive intervals. Some of these observations are presented in table 2.

Several facts are evident from these results. After a plant shows initial symptoms of infection, only a rough approximation can be made as to the expected progress of the disease unless the relative resistance of the host is known. A few resistant Red Currant plants appeared somewhat seriously affected a week after inoculation but in a few more days seemed to outgrow the symptoms and were soon apparently normal in development. Some of the wilt-tolerant Marglobe plants that seemed mildly diseased after 7 days, apparently succeeded in outgrowing this condition; although some plants remained mildly stunted and had not regained full vigor of growth 39 days after inoculation. In some other cases mildly diseased plants seemed to become normal and vigorous in growth, although at one time fairly seriously diseased. Marglobe plants that were severely diseased, after 7 days became steadily worse and finally died, although a longer period was required than in the susceptible variety Bonny Best. In all Bonny Best plants in which disease was notable as an outward symptom a week after inoculation, death occurred in a few days. It will be noted that in some cases Bonny Best showed complete wilting as early as 4 days after inoculation, a period much shorter than was ever observed in Marglobe. Incidentally, these data show that the period of a week allowed to elapse after inoculating plants before recording the observations is about the most satisfactory time to observe the relative differences in disease effects.

It is, of course, necessary in every test to grow noninoculated plants and to use an adequate control or standard series of treatments of varieties for comparative purposes. In studying differences in resistance of tomato, strains of known susceptibility are inoculated and grown along with the others of unknown reaction. In studying differences in pathogenicity of Fusarium isolates, susceptible or intermediately resistant plants are inoculated with cultures of known virulence and grown along with plants inocu-

TABLE 2.—*Disease progress in single plants in tomato varieties after infection with Fusarium bulbigenum var. lycopersici of highly virulent form. Evaluations based upon outward appearance only, range from 0 = no apparent above-ground symptoms, to 15 = collapse following severe wilt*

Experiment	Tomato plant variety[a]	Disease evaluation judged on outward appearances after							
		2 days	4 days	7 days	10 days	17 days	28 days	36 days	39 days
A	Bonny Best	0	0	5	6	10	15		
	Marglobe		0	5	5	5	5	M[b]	M
B	Bonny Best	3	10	15					
	" "	4	10	15					
	Marglobe	0	2	8	9	15			
	Red Currant	0	0	4	M	M	H[c]	H	H
	" "	0	0	5	M	M	H	H	H
	" "	0	0	4	M	M	H	H	H
C	Bonny Best			10	15				
	" "	0	6	10	15				
	Marglobe		0	7	8	15			
	"	0	0	8	9	15			
	Red Currant		0	3	M	H	H	H	H
	" "	0	0	3	M	H	H	H	
D	Red Currant	0	0	2	M	H	H	H	H
	" "		0	0	M	H	H	H	H
E	Bonny Best	0	3	5	7	15			
	" "	0	6	7	10	15			
F	Bonny Best		9	9	15				
	Marglobe		0	3	3	3	5	M	H
	"		4	5	5	6	M	M	H
	"		0	5	6	6	6	M	M
	Red Currant		0	0	H	H	H		
G	Marglobe		0	8	8	10	15		
	"		0	6	8	8	9	S[d]	S

[a] Bonny Best, susceptible to Fusarium wilt, Marglobe, tolerant or partly resistant, and Red Currant, resistant.

[b] M = Mildly stunted by disease. No obvious yellowing or wilting of leaves.

[c] H = Healthy appearance; plant developing flowers and fruits as successfully as noninoculated disease free plants.

[d] S = Severely stunted by disease; plant "outgrowing" yellowing and wilting previously noted.

lated with the unknown fungus cultures for a basis upon which to judge relative pathogenicity.

In the case of uncontrollable variation in environmental conditions the progress of a known severely pathogenic type of organism on fully susceptible plants will serve as a guide to the investigator. The writer found that if all seedlings are pulled and evaluated 2 or 3 days after complete wilting of the majority of fully susceptible plants inoculated with a virulent strain of *Fusarium* (evaluation 10 at wilting time, evaluation 15 when pulled) significant differences will be apparent among the various treatments, if differences are to be found.

SUMMARY

A laboratory-greenhouse technique is described for the study of both the pathogenicity of the tomato-Fusarium-wilt organism (*Fusarium bulbigenum* var. *lycopersici* (Brushi) Wr. and R.) and the relative wilt resistance of tomato strains and varieties.

The soil used was a prepared sand-soil mixture that was autoclaved before each trial. Tomato seedlings were grown in sterilized soil from 4 to 6 weeks in a warm greenhouse, and inoculum was made from *Fusarium* cultures grown on a liquid medium.

At time of inoculation, roots of seedlings were washed, dipped in inoculum, and planted. Soil temperatures were maintained around 25° to 28° C., and air temperatures in the greenhouse from 24° to 30° C. Plants were watered carefully to avoid excessively dry or wet conditions.

The period required for growing plants in test beds to get fungus-host reactions averaged about one week.

A system of numerical disease evaluation in plants is described and illustrated by diagrams by which it is possible to classify increasing severity of the disease by increasing numbers, with 0 representing no apparent infection, and 15 representing the earliest death from Fusarium infection.

Repeated trials have shown that this technique can be depended upon for determination of relative resistance or susceptibility to Fusarium wilt in strains of tomato and of relative virulence of strains of the disease organism.

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BACTERIAL LEAF SPOT OF DIEFFENBACHIA

LUCIA MCCULLOCH AND P. P. PIRONE

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Dieffenbachia picta Schott is an attractive ornamental foliage plant already rather widely grown and of increasing importance in the florists' trade (Fig. 1, A). The broad green leaves, attractively marked with pure white, its graceful habit of growth and its tolerance of the usual conditions of shade and dry atmosphere of dwellings, combine to make it a handsome and a useful ornamental plant. Recently a disease has caused loss and concern for the future usefulness of these plants. Parts of leaves, the entire leaf, and often all leaves become discolored, wilted, and finally killed.

The junior writer made the first studies of the disease and determined its bacterial nature.[1] No other reference to this disease has been found.

DESCRIPTION OF THE DISEASE

All parts of the leaf blade except the midrib are susceptible. Petioles and stems are not infected. Leaf spots are at first tiny, translucent specks, but these enlarge to several mm. or even 1 cm. in diameter (Fig. 1, B). They are more or less circular to elongated and yellow or orange-yellow. While the general effect is that of a yellow spot, close examination shows a central area of dull watery green surrounded by a border of orange-brown. The extreme outer margin is irregular (Fig. 1, C). By transmitted light the center is pale greenish-yellow and translucent while the border is a bright yellowish-orange color. Lesions near a vein fail to expand beyond it (Fig. 1, D, A). Usually a number of reddish-brown specks are noted in a ring between the center and the outer margin of the lesions or else irregularly distributed. Under unfavorable conditions such as too dry an atmosphere, the lesions cannot enlarge and therefore remain as small, dry, red-brown specks on the leaf.

When the lesions are numerous and conditions favorable for the parasite, they coalesce to form rather large areas. Such areas become yellow, wilted and dry, and parts of the leaf beyond the infected areas become yellow and die (Fig. 1, B). Even a few strategically located lesions can cause death to large areas of the leaf.

The dead leaves are dull tan or light brown, thin and tough (not brittle), and the lesions are dark brown by reflected light; pale green and translucent by transmitted light. There is considerable exudate consisting mostly of bacteria from the lower surface of the lesions. When moist, this exudate is soft; when dry, it is a somewhat waxy, silvery-white, thin layer covering the lesion. In advanced stages some exudate occurs on the upper surface also.

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[1] Pirone, P. P. Bacterial leaf spot of *Dieffenbachia*. (Abstract.) Phytopath. 29: 19. 1939.

THE CAUSAL ORGANISM

Isolation and Inoculation

The parasite has been isolated and its pathogenicity proved by both writers. Koch's postulates were fulfilled with 7 isolates. For isolation, young, unbroken lesions were sterilized 3 to 4 minutes in $HgCl_2$, 1–1000 to eliminate secondary bacteria, which are usually rather numerous on the leaf. Unless destroyed these saprophytes grow rapidly, often entirely preventing the development of the parasite, which is a rather slow growing organism.

Using the yellow growth produced by this bacterium, both authors have inoculated *Dieffenbachia* plants and secured lesions and disease symptoms identical in appearance with the natural infections. Infections were readily secured by spraying the leaves, with the bacteria suspended in water. The spray was applied to the lower surface as the infection occurs mostly, perhaps exclusively, through the stomata which are developed only on the under surface.

The symptoms of the disease, either outdoors or in the greenhouse, may appear in from 15 to 18 days, and the infection may never be serious. With a warm (70° to 75° F.), moist atmosphere, fairly constantly maintained, the lesions are visible in 7 to 8 days as tiny, translucent specks, which continue to develop and produce the usual disease symptoms. When conditions continue favorable for the parasite, large areas or even whole leaves become yellow and dead in 3 to 4 weeks. Such leaves wilt and droop on the stems. New leaves developing after inoculation do not necessarily become infected. The salability of the plant is greatly reduced, however, by the loss of even a few of the lower leaves.

Young lesions on *Dieffenbachia* leaves were fixed, sectioned, and stained. These show stomatal infections and bacteria spreading considerably in the lower cells of the leaf before those of the upper surface are invaded.

Dracaena fragrans Ker-Gawl plants were also inoculated with the *Dieffenbachia* leaf-spot bacteria. A considerable number of lesions developed, but most of them remained in the tiny, translucent speck stage. Only four reached a diameter of 2 mm. Where the small lesions were numerous and close together, they caused yellowing of the adjoining leaf tissue. The infection as a whole was negligible. Under some conditions more serious infection might occur on *Dracaena*. *Iris* and *Convallaria* plants were inoculated but no infections resulted, whereas parallel inoculations under identical conditions caused severe infection on *Dieffenbachias*.

MORPHOLOGY

The bacteria[2] are slender, round-ended rods. Young (2 to 4 days old) beef-agar cultures stained with carbol fuchsin, show single rods 0.3 to 0.4 µ wide by 0.9 to 2.8 μ long (the longer individuals may be paired rods but without a visible division mark). Others, plainly in pairs, were 2.0 to 2.8 μ long. The majority of definitely single rods are 0.3 to 0.4 μ by 1.0 to 1.5 μ. Capsules are absent or inconspicuous in beef agar but in beef plus starch, and in potato dextrose agar, and in synthetic agars plus dextrose or saccharose, the capsules are well developed and stain well with Ribbert's[3] dahlia stain.

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[2] In most of the tests, seven different single colony isolations of the bacterium were used to obtain the descriptions. Never less than two were used.

The flagella are single, polar, 2 to 3 times as long as the rod, mostly wavy. The Casares-Gil[4] method was used with 2-day-old beef agar cultures of the bacteria. No spores or involution form were observed. The bacteria are Gram-negative and are not acid-fast.

CULTURAL CHARACTERS

Colony growth is slow at 23° to 25° C. on beef-peptone agar[5] plates inoculated directly from leaf spots or from a culture. Tiny white specks visible in 3 to 5 days slowly enlarge, become pale yellow, then Massicot Yellow or Naples Yellow.[6] In 6 to 7 days well isolated colonies are 2 to 4 mm. in diameter. They are circular, entire, flat, smooth, thin and translucent. Young colonies usually have concentric striations, which later disappear. With slight magnification there is noted a fairly wide transparent border and a denser, coarsely granular center showing faint, irregular cracks which probably indicate the beginning of a later mottled structure. In the mottled stage the surface remains smooth but the interior has irregular, opaque areas. This mottling is usual in old colonies but it does not always develop. In crowded plates the surface colonies remain small, pale yellow, thin and transparent. Submerged colonies are translucent, rather granular, spherical, spindle-shaped or triangular.

Beef agar slants inoculated from beef broth or other liquid culture develop a smooth, thin, transparent layer of growth. If inoculated from agar cultures the butyrous growth does not spread evenly and a thicker, irregularly contoured, translucent growth develops. If there is water at the base of the slant, the bacterial growth ends abruptly at its surface and even after the water has evaporated, the lower limit of growth remains at the original water level. This definite lower limit of growth in beef agar slants is a constant character. All agar cultures 3 to more weeks old are transparent.

In beef agar stabs there is a trace of growth in the upper 2 mm. The bacterial growth spreads slowly and seldom entirely covers the surface of the agar.

Beef extract agar permits slightly less growth, which is less yellow than on the beef-infusion agar.

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[3] Ribbert. Zur Färbung der Pneumoniekokken. Deut. Med. Wochnschr. 11: 136. 1885.

[4] Galli-Valerio, B. La méthode de Casares-Gil pour la coloration des cils des bactéries. Centbl. Bakt. Bd. 76. Heft 2/3. Abt. I. 1915. p. 233–234.

[5] Unless otherwise stated, all beef media were made with beef infusion and with a pH value between 6.5 and 7.0.

[6] Ridgway, R. Color standards and color nomenclature. 43 pp. (Washington). 1912.

On all the solid beef agar media the growth is soft, butyrous in texture. In cultures 2 to 3 weeks old and in those long in artificial media and often transferred, the growth is slightly viscid or slimy. Old, dry cultures have a slight iridescence. There is no fluorescence in any of the media tried.

Beef broth cultures at favorable temperatures have a thin, uniform clouding. Undisturbed cultures eventually have heavier clouding at the surface and irregular, patchy yellow rims. Pellicles form less frequently than rims. Tiny crystals are numerous in the rims and pellicles. Sediment is scanty to moderate, loose and flocculent, easily dissolved by shaking.

Beef agar or broth plus 0.2 per cent starch or 1 per cent dextrose is more favorable for this organism than the usual beef agar.

Beef gelatin plates are quickly liquefied at 24° C. Tube cultures liquefy the upper 10 mm. in 3 days, afterwards the action is slower with entire liquefaction in about 5 weeks. Liquefaction is stratiform, the liquefied gelatin is very slightly clouded, there is no surface growth, such as pellicle or rim, and the sediment is abundant, yellow, fine-grained, and easily dissolved.

On blood-serum slants growth is good and, as in beef agar, the bacterial growth ends abruptly at the water level. Liquefaction was complete in 3 weeks at 28° C.

Potato dextrose agar with a pH value of 5.6 to 6.0 produces an abundant, thick, smooth, translucent growth, which extends into the water and as the agar shrinks, spreads to all the exposed areas, entirely filling the lower part of the tubes.

Potato cylinders are not particularly favorable for growth. A thin, wet, yellow layer forms on the slanted surface, never spreading over the edge or below the water level. The growth is so thin that the texture of the potato shows through it. The color of the growth is Massicot to Wax Yellow.

Uschinsky's solution clouds faintly, then growth ceases. No growth occurs in Cohn's solution.

The bacteria grew well in tryptophane broth and cultures tested by the Ehrlich-Böhmé method seemed to have indol present. Other cultures subjected to the Goré test gave no indol reaction.

Hydrogen sulphide and ammonia are produced to a moderate degree.

Nitrates are not reduced. Cultures were made in several nitrate media, including that recommended in the Manual of Methods[7] for difficult cases. Tests made with the sulphuric acid-α-naphthylamine acetic acid on the 12th and 16th days gave no indication of reduction.

Starch is only moderately hydrolyzed. Streak inoculations on beef agar plus 0.2 per cent starch produced good growth, and in 7 days a zone 6 to 9 mm. wide on either side of the growth was free from starch.

Milk is slowly peptonized. Except for the presence of thin yellow rims there is no visible change for several days at 30° C. In 3 weeks a small amount of soft jelly-like curd remains at the base of the clear whey. Numerous tyrosin crystals are present in cultures 4 to 6 weeks old.

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[7] Society of American Bacteriologists. Committee on bacteriological technique. Pure-culture study of bacteria. 1923 to May, 1939.

Litmus in milk shows some reduction in 4 days, and complete reduction in 8 days. Some returning color is evident in 27 days. Tyrosin crystals form later.

Fermentation and acid reaction at 30° C. are fairly prompt and strong on dextrose, saccharose, galactose, and levulose; good growth and acid reaction occur in 8 to 10 days. On lactose and glycerol, growth is slow but eventually good and in 20 days acid reaction is definite. On maltose and mannitol growth is even slower than on glycerol but in 5 weeks (the cultures had to be moistened occasionally) there was a fair amount of growth but no indication of acid. Glycerol and mannitol were the least favorable, and cultures on these often failed to produce any growth. On all these media the growth is thick, smooth, colorless at first and later yellow. A peptone-free synthetic agar with brom cresol purple as indicator and 1 per cent of the various carbohydrates was used in the above tests. No evidence of gas was observed at any time in deep-stab or shake-agar cultures. In 1 of 5 tests there was a slight reduction of the indicator (brom cresol purple).

The optimum pH value for growth on beef media is 6.4 to 6.8 and the limits for growth are 5.4 and 8.0. The pH value of the media was determined immediately before inoculation. The cultures were observed for 4 days.

The organism proved to be aerobic.

VITALITY

Beef agar and beef broth cultures remain alive for at least 4 months at room temperature, but with evidence of reduced vigor. Similar cultures kept at 4° to 5° C. for 6 months retain their original vigor. Transfers made to potato-dextrose agar, from either old or young cultures, grow better than if made to beef agar. Some old cultures fail to renew growth in beef media, but do grow in potato-dextrose agar. Beef agar cultures kept at – 17° to – 20° C. were alive and grew well in transfers made 7, 24, and 63 days and 6 months later.

Freshly inoculated beef broth and beef agar tubes were frozen for 7 days at – 5° C. without injury to the vitality of the bacteria. Others at 0° for 30 days grew promptly when removed to 30° C.

The optimum temperature for growth is 30° to 31° C. At 33°, growth during the first hours is more rapid, but after 3 days is less than at 30° to 31°. The minimum temperature for growth is about 5°; maximum between 37° and 38°; the thermal death point is 48° (beef broth, freshly inoculated, held at desired temperatures for 10 min. under controlled temperature conditions).

The organism has only slight resistance against desiccation. Beef-agar cultures diluted in water and dried on cover glasses were dead in 6 days or less. If diluted in beef broth vitality was retained for 22 days. When 12 whole colonies were transferred from a dry, 35-day-old beef-agar plate to potato-dextrose slants, no growth resulted.

No growth occurred on freshly inoculated beef-agar plates exposed to

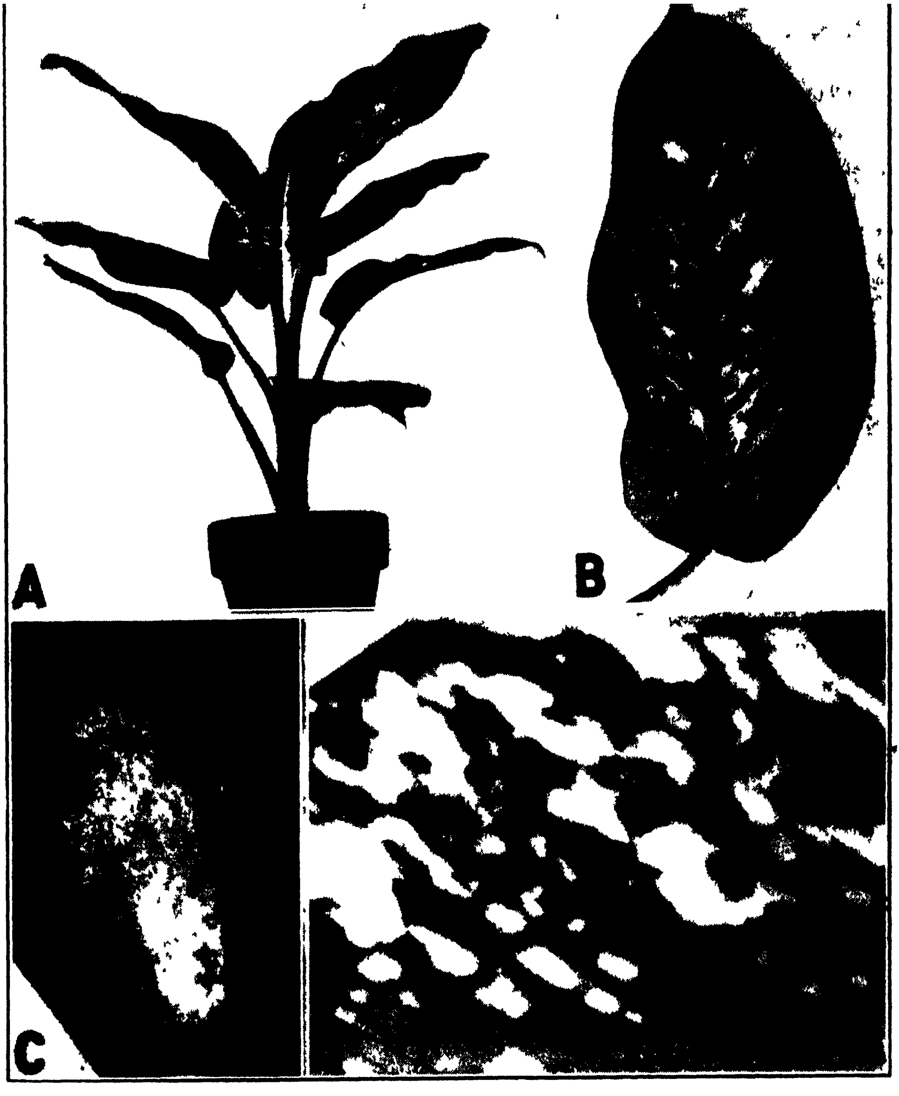

FIG. 1. A. Normal *Dieffenbachia* plant. × ⅛. B. Leaf with bacterial leaf spot. × ⅓. C. Single lesion. × 5. D. Bacterial lesions on leaf. × 2½.

direct sunlight for 5 minutes (temp. 3° to 5° C.). On control areas protected from the light, growth was abundant.

Bacteria from young (2- to 3-day-old) beef-agar cultures survive, apparently without loss of vigor, for at least 48 hours in either tap or distilled water. Bacteria from a 6-week-old beef agar failed to grow after only 25 minutes in water.

In a 1 to 100,000 solution of $HgCl_2$ all the bacteria were dead in 5 minutes. Controls from the same culture remained alive in water for 48 hours or more.

CONTROL

Separate the infected plants from the healthy plants. If possible, lower the temperature since high temperatures (85° to 90° F.) encourage the growth of the bacteria. Avoid syringing, as this practice tends to spread the bacteria from plant to plant. These methods were successfully tried on a large number of *Dieffenbachia* plants.

Specimens of infected *Dieffenbachia* leaves have been deposited in the mycological collection of the Bureau of Plant Industry, Washington, D. C.

TECHNICAL DESCRIPTION

Bacterium dieffenbachiae, n. sp. (*Phytomonas dieffenbachiae*, n. sp. (Bergey *et al.*).) Short rods, ends rounded, single and in pairs. Size 0.3 to 0.4 μ by 1.0 to 1.5 μ for single rods. Motile; single, wavy polar flagellum three to four times the length of the rod. Capsules very indefinite in beef media cultures but well developed in cultures containing dextrose or starch. No spores or involution forms. Aerobic. Yellow growth on culture media. Gelatin is liquefied. Blood serum is liquefied.

Ammonia and hydrogen sulphide are produced. No indol. Nitrates are not reduced.

Slight growth in Uschinsky's solution. No growth in Cohn's solution. Milk is slowly peptonized. Litmus in milk is reduced.

Starch is only moderately hydrolyzed. Acid reaction from dextrose, saccharose, lactose, galactose, levulose and glycerine. Grows but does not produce acid on maltose and mannit. The optimum pH for growth is 6.4 to 6.8. The maximum temperature is between 37° and 38°; minimum about 5°; optimum 30° to 31° C; thermal death point 48°. Only slightly resistant to sunlight and to drying. Gram negative. Not acid-fast. Pathogenic to *Dieffenbachia picta* and to a slight extent to *Dracaena fragrans*, producing a severe leaf spotting and destruction of the leaves of *Dieffenbachia*.

SUMMARY

A bacterial disease of *Dieffenbachia picta* Schott is described. The causal organism is a bacterium which has been isolated and its pathogenicity proved by inoculation experiments. The bacteria enter the leaf tissue through the stomata. Unsightly yellow-brown spots develop, which, when numerous, result in yellowing, wilting, and death of the leaves. The morphological, cultural, and physiological characters of the pathogen are described. Methods of control and prevention are suggested.

The name *Bacterium dieffenbachiae* is suggested for the pathogen.

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PHYSIOLOGIC RACES OF WHEAT LEAF RUST INVOLVED IN THE 1938 EPIPHYTOTIC

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(Accepted for publication June 14, 1939)

INTRODUCTION

The unusually destructive epiphytotic of wheat leaf rust (*Puccinia rubigo-vera tritici* (Eriks. and Henn.) Carl. = *P. triticina* Eriks.) in 1938, has been the subject of a number of communications to the Plant Disease Reporter and the Cereal Courier. In view of the severity of the disease in Oklahoma (1), it appeared desirable to undertake a study of the biology and epiphytology of leaf rust under Oklahoma conditions. Brief accounts of one phase of the study have been published (2, 3). The present paper deals with the physiologic races of leaf rust involved in the 1938 epiphytotic and with their infectivity on commercial wheats and native grasses. The writers are indebted to C. O. Johnston, who provided seed of the differential wheat varieties used in race analysis and offered constructive criticisms on interpretation, and to M. Newton and T. Johnson, whose suggestions were a material aid in the work.

MATERIALS AND METHODS

During the 1938 growing season about 150 collections of leaf rust were made at scattered points in Oklahoma. To these were added collections made in the vicinity of Stillwater at weekly intervals from November, 1938, throughout the winter. Most of these collections were analyzed for race by the usual methods (6). Seedlings of Turkey, Blackhull, Red Cross (Michigan Amber), Little Club, and Malakof wheats were used as stock varieties for culture, isolation, and multiplication of the individual rust races. On one or another of these varieties, each collection was carried through one or more single-pustule transfers before being inoculated on the differential wheat varieties. Cotton-plugged lamp chimneys served as moist chambers. The work was in progress only during the period in which it was possible to maintain an average greenhouse temperature of 65° F. with daily fluctuations that did not pass beyond the range 50° to 80° F. Light was of moderate intensity. The seedlings were grown in 4-in. pots in a rich loam, and were inoculated on the prophyllum at the time the first true leaf was well developed. Under these conditions the wheat grew well, and strong infections were readily produced on susceptible plants.

Readings of infection types usually were made on the 10th day after inoculation, but occasionally later, in midwinter. Infection types were determined by reference to the descriptions of Johnston and Mains (6). In addition, specimens showing the various infection types were preserved in Vacha's solution[1] and sent to M. Newton and T. Johnson, whose readings of the specimens differed in no essential from those of the writers. The races of leaf rust were identified by reference to the register of leaf-rust races of Humphrey *et al.* (5).

EXPERIMENTAL RESULTS

Physiologic race 13 appears to have been the most prevalent and widespread race in Oklahoma during the 1938 epiphytotic. It was identified 49 times in 98 spring collections. Races 19 and 77 were next in frequency, occurring 10 and 8 times, respectively, and race 9 was found in 6 cases. The remaining races appeared to be of only minor importance in the epiphytotic: race 20 was found 5 times; race 5, 4 times; race 18, 3 times; races 2, 52, and 54, twice each; races 12, 28, 31, 33, 43, 58, and 68, once each.

In the fall and winter collections, race 13 occurred 9 times, races 19 and 77, once each.

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[1] Vacha, G. A. Formulae for preserving colors in fruits and flowers. Dept. Pl. Path., Minn. Agr. Exp. Stat. (Mimeographed, bears no date).

TABLE 1.—*Leaf-rust resistance displayed by certain breeding wheats when subjected to the leaf-rust races prevalent in Oklahoma in 1938*

Wheat variety	C.I. no. or hybrid no.	Reaction of juvenile plant[a] to race						Reaction of adult plant to mass infection at	
		13	19	9	77	5	2	Jointing	Heading
Quivira	8886	4	4	4	3–	0–2+	3	2–3	2
Turkey Sel.	10100	4	4	3	3	4	4	2–3	3
Kanred × Hard Federation	10092	3	3	3	3	1–3	3–	0(–3)	0(–3)
Prelude × Kanred	11591	4	4	3	3	4	3	0(–3)	0(–3)
Chiefkan	11754	4	?	4	4	4–	4	3	(0–)3
Ill. No. 1, W–38	Kans. 37FN25	4	3	3	4	4	4	2–3	3–4
Kawvale × Marquillo	Kans. 37FN748	0	0–2	0–1	4–	4	3	0	0–2
Kawvale	8180	0–3	1	3	3–4	4	3–4	1(–3)	3
Kharkof	1442	4	4	4	4	0–2	3–4	3	3
Kawvale × Tenmarq	11750	1(–3)	1	0(–3)	4	4	3–4	2	3
Mediterranean	3332	1	1	1	4	4	4	(2–)–4	3
Hope × Hussar	11835	1	1	3	1	0–2	3–4	0(–3)	0–2
Hope × Kawvale	Kans. J361606	0	0	1	3–4	4–	4	0–4	3
Mediterranean 3015–63 × Hope	Tex. 41–17–3	3	3	3	4	4	4–	0–4	0–3
Mediterranean 3015–63		3	(1)–4	3	4	4	3–4	3	0–3
Marquillo × Oro	FN787–3	4	4	4	3+	4	4	(0–2)–3	2–3
Ceres × Hope-Florence	11708	0	0	0–3	1–3	3	1–3	0–4	0–3
Oro × Tenmarq	11673	4	4	4	4	3	4–	(0–)3	0–3

[a] Reaction types as described by Johnston and Mains (6). Types in parenthesis were present but only in relatively small amount; *i.e.*, 0–2(–4) indicates that the majority of the infections were of types 0, 1, and 2, although a few type-4 pustules were present.

Each of the group of superior-germplasm wheats, which had been distributed to cereal investigators in a number of States, was inoculated individually in the seedling stage with each of the leaf-rust races 13, 19, 9, 77, 5, and 2. The infection types were recorded and the plants grown to maturity in 6-in. pots, 5 plants to a pot. At the time of jointing, when the plants were too large to be inoculated individually, the whole group of 300 pots was subjected to a mass inoculation with a pooled suspension of the 6 races mentioned above. The infection was increased to proportions of an epiphytotic by daily syringing in the evening. Readings of infection types were taken at the jointing stage and again at heading. Those varieties showing rust resistance at the time of one or more of these readings are listed in table 1. The remaining varieties indicated no resistance, *i.e.*, showed only infection types 3 and 4 throughout their entire development. This group of entirely susceptible wheats comprised: Minhardi, Lutescens, Yogo, Minturki × Marquis, Early Blackhull, Tenmarq, Blackhull, Clarkan, Marvel, Marquillo × Tenmarq, Turkey (S. Dak. 144), Cheyenne, Ridit × C. I. 8033, Oro × Mont. No. 36, Wheat × Rye (Meister), Ridit, Oro, Turkey Sel., Martin × Tenmarq, Akron Sel. No. 7, *T. timopheevi*, Forward, Valprize, Kanhull, Oro, Albit, Hybrid 128, and Deluxe Chiefkan.

Of the 50 breeding wheats tested, the 2 showing greatest resistance to leaf rust at all stages were Kawvale × Marquillo and Hope × Hussar.

Leaf rusts were very common on grasses in Oklahoma during 1938. The literature affords little indication that the leaf rust of wheat can propagate on wild grasses (4, 7). It was felt desirable, however, to carry out cross-infection tests with the native grasses of Oklahoma in connection with the problem of the oversummering of the rust. Seventy-five species of native Oklahoma, Texas, and Kansas grasses were grown to a size suitable for inoculation, and were then inoculated with a pooled suspension of urediospores of races 13, 19, 77, 9, 2, and 5. The grasses were species of *Agropyron, Agrostis, Andropogon, Astrebla, Bouteloua, Brachtypodium, Bromus, Buchloë, Chloris, Cymbopogon, Dactylis, Diarrhena, Elymus, Eragrostis, Erianthus, Festuca, Leptochloa, Muhlenbergia, Panicum, Pappophorum, Paspalum, Phalaris, Poa, Schedonnardus, Setaria, Sphenopholis, Sporobolus, Stipa, Triodia, Tricachne,* and *Valota.*

Of the 75 species of inoculated grasses, only one, *Agropyron trichophorum* (Link.) Richt., showed infection with production of urediospores. In this case, however, although the pustules appeared to be fairly well developed, the spores were incapable of reinfecting wheat. Parallel inoculations of the same rust pool on susceptible wheats at the same time and under similar conditions, produced good infections.

DISCUSSION

Our tests indicate that race 13 was the most prevalent in Oklahoma during the 1938 epiphytotic, while races 19 and 9 were present but less prevalent than race 13. C. O. Johnston advises us that race 9 was most frequently identified in Kansas in the same year. It is believed that this indicates no discrepancy. Races 9, 13, and 19 are very similar, as indicated by their reactions on the differential wheat varieties in table 2, differing only in their reactions on the varieties Carina and Hussar, both of which are inclined to variable reaction in the greenhouse. It will be noted that race 13 is characterized by somewhat higher infection types than race 9, which indicates that our experimental conditions were favorable for leaf-rust analysis. The tests were usually so conducted that 12 to 20 collections were analyzed simultaneously; and it is noteworthy that races 13 and 9 were rarely identified at the same time. Rather, a group of race 9 identifications occasionally appeared together. When race 9 and race 13 were cultured side-by-side on breeding wheats, some difference could be detected between the two races, but this was slight (Table 1). These comments apply equally well to race 19.

TABLE 2.—*Reactions of certain leaf-rust races on the 8 differential wheats*

Race	Malakof	Carina	Brevit	Webster	Loro	Mediterranean	Hussar	Democrat
9	4	1–2	1–2	4	4	0–1	1–2+	0–1
19	4	4	2	4	4	0	1	0
13	4	4	2	4	4	0	4	0
5	4	0	0–1	0–1	0–1	4	0–2+	4
15	0	0	0–1	0	0–1	4	0–1	4
77	4	4	4	4	4	4	4	4

It is believed, from these considerations, that races 13, 19, and 9 are so closely related that they may be considered as largely environmental variations of the same race. The differences between them are of hardly greater order than the differences between any two collections of the same race when two such collections are used to infect a large number of wheat varieties. The contention of Johnston and Mains that race 9 appears well adapted to conditions in the Southwest (6) applies equally well to races 13 and 19, since these were readily collected in Oklahoma during the winter of 1938–39 after severe freezing.

The same relation appears to exist between races 5 and 15 as between races 13, 19, and 9, with race 5 distinguished only by its stronger reaction on Malakof, although both races 5 and 15 are entirely distinct from the race-9 complex. Our determinations in this group were restricted to race 5, while Johnston's were largely of race 15. Here, also, the same races were probably involved in both Kansas and Oklahoma, the difference being due to minor variations in the greenhouse environment.

Race 77 infects all 8 differential wheat varieties with a type-4 reaction, thus representing the most highly infectious of the 109 described physiologic races of leaf rust. It appears to have a good degree of hardiness, since it was collected in midwinter after severe freezing. On the other hand, its incubation period was usually 1–2 days longer than that of race 13, a fact that might limit its distribution in the field in competition with race 13, which is equally infective to ordinary commercial wheats. In breeding for leaf-rust resistance, however, race 77 offers potentialities of future trouble, because its ability to infect all 8 differential varieties, which afford a wide variety of defensive mechanisms in wheat, indicates that race 77 is equipped with an equally wide variety of the factors that can overcome these defense mechanisms.

Our inability to propagate leaf-rust on 75 species of native grasses is in accordance with the findings of others in this regard (4, 7) and affords evidence toward the view that Oklahoma is dependent upon outside sources for fall inoculum (2, 3).

SUMMARY

Analysis of 98 leaf-rust collections from Oklahoma in 1938 indicates that the principal rust races involved in the 1938 epiphytotic were, in decreasing order of prevalence, 13, 19, 77, and 9. Races 13, 19, and 9 are considered variants of the same race, their slight reaction differences being largely due to the effect of environment on the sensitive wheat varieties, Carina and Hussar. Similarly, races 5 and 15 are considered variants of the same race. Race 77, which was occasionally identified, is considered as potentially dangerous because of its extensive equipment of the factors which can overcome many resistance factors in wheat. At present, however, it does not compete successfully with races 13, 19, and 9, which equally well infect commercial wheats, and have a slightly shorter incubation period.

Of 50 wheat varieties of superior germplasm inoculated with leaf-rust races 13, 19, 9, 77, 5, and 2, both individually and collectively, a number showed resistance to one or more of these races. The two wheats showing the greatest resistance from seedling stage to maturity were the hybrids Kawvale x Marquillo, and Hope x Hussar.

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BACTERIAL LEAF SPOT OF MAPLE[1]

P. A. Ark

(Accepted for publication May 9, 1939)

A bacterial disease of maple was recently reported by Ogawa[2] in Japan. It occurs principally on *Acer trifidum* Hook. et Arn., but 13 other species of *Acer* are also susceptible to *Phytomonas acernea,* the causal organism. The disease sometimes occurs in epidemic form. The symptoms are chiefly expressed on leaves and petioles, while occasionally there is a blighting of twigs. Affected leaves drop off and thus cause defoliation of the tree.

In California, a similar disease was observed for the first time in 1937, in a nursery in Berkeley. Seedlings of *Acer macrophyllum* Pursh. showed

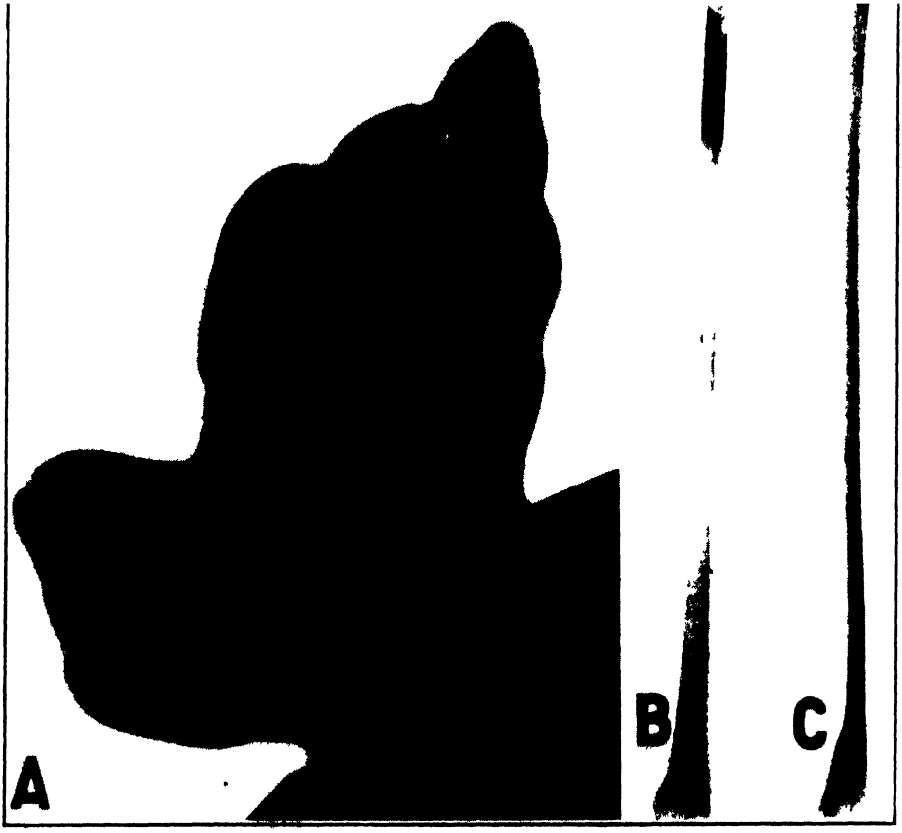

FIG. 1. A and B. Infections on *Acer macrophyllum* produced by spraying leaf with a suspension of *Phytomonas aceris:* A, leaf spot; B, petiole canker; C, check (petiole from a leaf of *A. macrophyllum* sprayed with distilled water).

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[1] Contribution from the Division of Plant Pathology, University of California, Berkeley, California. The assistance of nontechnical employees of the Federal Works Progress Administration is acknowledged.

[2] Ogawa, Takasi. Shoot drooping disease of *Acer trifidum* Hook. et Arn. caused by *Pseudomonas acernea,* n. sp. Annals Phytopath. Soc. Japan 7: 125–135. 1937. English summary.

numerous spots on leaves, varying in diameter from a pin point to ¼ of an inch. At first the spots are water-soaked, surrounded by a yellow zone; later, they turn dark brown or black. If the disease is very serious, the petioles and brackets may develop cankers (Fig. 1). The affected leaves may sometimes absciss. The disease occurs early in the spring during cool, damp weather.

When a leaf lesion is crushed on a slide in a drop of water, a discharge of numerous, very actively motile bacteria may be observed. Plating of the crushed tissue in beef-extract or potato-dextrose-peptone agar gives a pure culture of the causal organism.

The organism grows rapidly on beef-extract-peptone agar, producing visible colonies in 24 hours at 28° C. The colonies are grayish-white and, if numerous, may produce a slight fluorescence in the medium. The beef-extract-peptone broth is promptly clouded upon inoculation. The optimum pH lies between 5 and 7.5; no growth was observed at pH 4, and very slight

TABLE 1.—*Comparison of cultural reactions of Phytomonas acernea Ogawa and Phytomonas aceris*

Items compared	*Phytomonas acernea*	*Phytomonas aceris*, n. sp.
Size	0.5 μ–1.2 μ × 0.2 μ–0.6 μ	0.8 μ–2.5 μ × 0.3 μ–0.8 μ
Motility	By one polar flagellum	By one or two polar flagella
Gram reaction	Negative	Negative
Optimum temp.	32° C.	13–31° C.
Chromogenesis	Citron yellow	None. Media slightly fluorescent
Uschinsky's solution	Grows. Degree of growth not reported	Weak growth
Cohn's solution	" " " "	Good growth. Fluorescence
Gelatin	Liquefied	Liquefied
Milk	Cleared slowly without coagulation	Cleared without coagulation
Nitrates	Reduced	Not reduced
H_2S	Positive	Negative
Starch	Not hydrolyzed	Not hydrolyzed
Indole	Not reported	Negative
Arabinose	Not reported	a. + b. –[b]
Dextrose	+ (slight)[a]	+ +
Dulcitol	Not reported	+ +
Galactose	+ (slight)	+ +
Glycerine	+ (slight)	+ –
Lactose	+ (slight)	+ +
Levulose	+ (slight)	+ –
Maltose	+ (slight)	+ +
Mannite	+ (slight)	+ +
Raffinose	Not reported	+ +
Sucrose	+ (slight)	+ +
Xylose	Not reported	+ +

[a] In peptone water.

[b] a. In a synthetic medium composed of:

$MgSO_4 \cdot 7\,H_2O$	0.02
$CaCl_2$	0.01
NaCl	0.01
$FeCl_3 \cdot 6\,H_2O$	trace
$(NH_4)_2SO_4$	0.20
Distilled water	100 cc.

b. In Dunham's peptone water.

growth in bouillon occurred at pH 10. The organism appears to be larger than the Japanese maple organism, described by Ogawa, being 0.8 μ–2.5 μ × 0.3 μ–0.8 μ, reduces nitrates, and produces hydrogen sulphide in lead acetate agar. While the Japanese organism is citron-yellow when streaked on an agar slant, the California maple organism is gray-white with a slight fluorescence in the medium. A comparison of cultural reactions is shown in table 1.[3] The above-mentioned differences, together with some differences in sugar reactions, suggest that the California maple bacterium is a different species, and, therefore, the name **Phytomonas aceris**, n. sp. is suggested.

The organism appears to be culturally different from *Phytomonas syringae* (van Hall) Bergey. Thus, while *Ph. syringae* does not produce any acid from lactose and maltose, the maple organism readily does so. Moreover, attempts to induce spots on maple leaves with certain strains of *Ph. syringae* were not successful.

A transfer of *Phytomonas aceris* has been sent to the American Type Culture Collection, Washington, D. C.

Acer macrophyllum is the most susceptible species to *Phytomonas aceris* under natural conditions. The following species of *Acer*, when sprayed with a suspension of *Ph. aceris* and incubated in a moist chamber, were found to be susceptible: *Acer circinatum* Pursh., *A. negundo* L., *A. negundo* var. *californicum* Sarg., and *A. palmatum* Thunb.

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NEW HOSTS AND DISTRIBUTION OF ELSINOË SOLIDAGINIS

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(Accepted for publication June 15, 1939)

In the first report concerning *Elsinoë solidaginis*, the cause of goldenrod scab,[1] the distribution was confined to the southern half of Florida. While the article was in press, however, record was obtained of its distribution in the United States Barbour Lathrop Plant Introduction Garden at Savannah, Georgia. This fact was introduced in the text (pp. 517–518) without further reference or indication on the distribution map.

These later records and others subsequently obtained from Florida, Georgia, and South Carolina are tabulated below in table 1, and the places where the fungus is now known to occur are shown on the accompanying map (Fig. 1).[2] The hosts of *Elsinoë solidaginis* previously given were *Solidago chapmanii*, *S. edisoniana*, *S. fistulosa*, and *S. sempervirens* growing

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[3] The bacteriological methods used were those described in the Manual of Methods for Pure Culture Study of Bacteria of the Society of American Bacteriologists.

[1] Jenkins, A. E., and Ukkelberg, H. G. Scab of goldenrod caused by *Elsinoë*. Jour. Agr. Res. 5: 515–525.

[2] *Loc. cit.* (see footnote 1, p. 524).

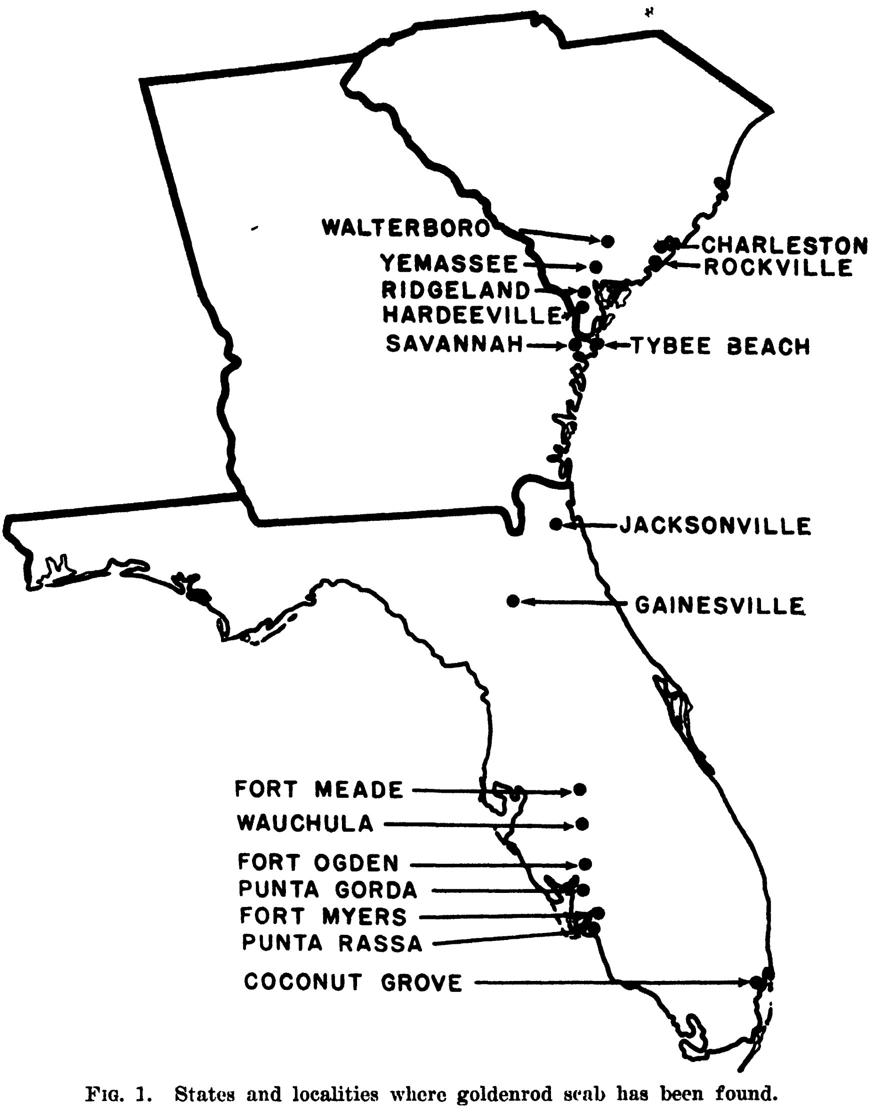

FIG. 1. States and localities where goldenrod scab has been found.

wild, and *S. elliottii, S. mirabilis,* and *S. leavenworthii* cultivated in the Edison Botanic Garden at Fort Myer, Florida. On *S. leavenworthii* only one instance of slight infection was recorded.

It would seem that the new localities represent the natural distribution of the *Elsinoë solidaginis* over a period of years prior to 1934, when plants were introduced from the Edison Botanic Garden to the Barbour Lathrop Plant Introduction Garden at Savannah. The plants that were introduced were cut back, so only a few inches of roots or stem remained, carefully inspected for freedom from lesions, and then the whole plant was disinfected. No infection was detected in the Garden during the growing season of 1934.[3]

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[3] See footnote 1.

TABLE 1.—*New records of goldenrod scab in northern Florida, Georgia, and South Carolina*

State	County	Place	Species of *Solidago* or *Brachychaeta*	Wild or cultivated	Date specimen was collected or infection observed	Collected or observed by—
Florida	Alachua	Gainesville	*S. fistulosa* Mill.	wild	Sept. 26, 1935	H. G. Ukkelberg
	Duval	Jacksonville	do	do	do	do
Georgia	Chatham	Savannah	*S. sempervirens* L.	do	Sept. 22, 1935	L. G. Polhamus and H. G. Ukkelberg[a]
do	do	do	*S. altissima* L.	cultivated[b]	Sept. 25, 1935	H. H. Hill and L. G. Polhamus
do	do	do	*S. leavenworthii* Torr. & Gray	do	do	do[c]
do	do	do	*S. serotina* Ait.	do	do	do[d]
do	do	do	*S. sempervirens* L.	wild	Oct. 11, 1935	L. G. Polhamus[c]
do	do	do	*S. fistulosa* Mill.	do	Oct. 29, 1935	do[d]
do	do	do	*S. altissima* L.	cultivated[b]	do	H. H. Hill
do	do	do	*S. edisoniana* Mackenzie	do	do	do
do	do	do	*S. elliottii* Torr. & Gray	do	do	do
do	do	do	*S. fistulosa* Mill.	do	do	do
do	do	do	*S. juncea* Ait.	do	do	do
do	do	do	*S. leavenworthii* Torr. & Gray	do	Nov. 13, 1935	do
do	do	do	*S. mirabilis* Small	do	do	do
do	do	do	*S. rugosa* Mill.	do	do	do
do	do	do	*S. sempervirens* L.	do	do	do
do	do	do	*S. serotina* Ait.	do	do	do
do	do	do	*S. bicolor* L.	do	Aug. 24, 1936	do
do	do	do	*S. brachyphylla* Chapm.	do	Sept. 1, 1936	do
do	do	do	*S. caesia* L.	do	do	do
do	do	do	*S. canadensis* L.	do	do	do
do	do	do	*S. petiolaris* Ait.	do	Sept. 4, 1937	do
do	do	do	*S. serotina gigantea* (Ait.) Gray	do	Autumn, 1937	do
do	do	do	*S. ulmifolia* Muhl.	do	do	do
do	do	do	*B. sphacelata* (Raf.) Britton	do	do	do
South Carolina	Charleston	Charleston[e]	*S. fistulosa* Mill.	wild	Oct. 19, 1935	Ralph Bailey
do	Colleton	Walterboro[e]	*S. altissima* L.	do	do	do
do	do	do[e]	*S. fistulosa* Mill.	do	do	do
do	Beaufort	Yemassee[e]	*S. sempervirens* L.	do	do	do
do	Jasper	Ridgeland[e]	*S. fistulosa* Mill.	do	do	do
do	Beaufort	Hardeeville	do	do	do	do
do	Wadmallaw Is.	Rockville	*S. sempervirens* L.	do	do	do

[a] Collected 10 miles from Savannah on road to Tybee Beach.
[b] In U. S. Plant Introduction Garden.
[c] Collected at Tybee Beach.
[d] Collected near U. S. Introduction Garden.
[e] Near highway U. S. 17.

As table 1 shows, however, infection was present in the Garden the following season, and it was also observed on wild goldenrod growing near the garden and also 10 miles distant (Table 1).

Among the species of goldenrod from the Introduction Garden listed in the table are 10 species and one variety on which the *Elsinoë solidaginis* has not previously been reported, as follows: *Solidago altissima, S. bicolor, S. brachyphylla, S. caesia, S. canadensis, S. juncea, S. rugosa, S. petiolaris, S. serotina, S. serotina gigantea,* and *S. ulmifolia.* Among these species the most severely affected in the Garden at Savannah has been *S. serotina.* Under the same conditions certain strains of *S. leavenworthii* appear to be almost entirely resistant. It will be noted that wild plants of *S. altissima* in South Carolina also were infected.

On a specimen of *Solidago fistulosa* sent from Savannah, Georgia, Nov. 13, 1936, by H. G. Ukkelberg, the perfect stage of the *Elsinoë* was present in abundance (Fig. 2).

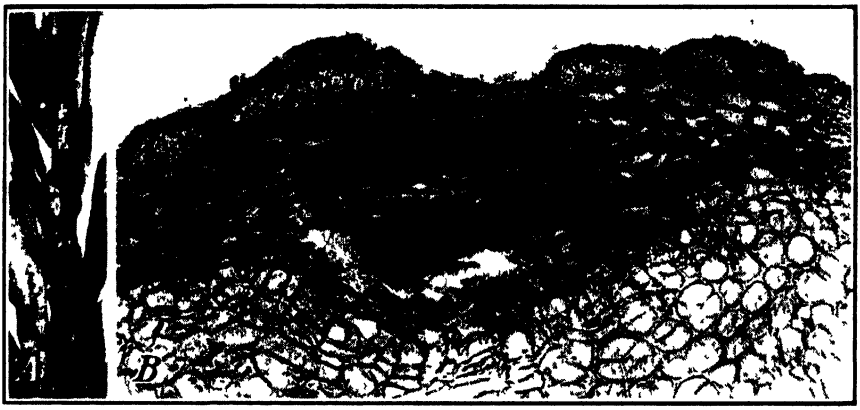

FIG. 2. A. Scab lesions on stem of *Solidago fistulosa.* B. Section of a lesion showing the ascigerous stage fruiting on the periphery. Material from United States Barbour Lathrop Plant Introduction Gardens, Savannah, Ga., 1936, H. G. Ukkelberg. A, ×1; B, 300.

Plants of a local composite, *Brachychaeta sphacelata,* were introduced into the Garden at Savannah. These became severely attacked by the scab, thus adding a new genus and species to the list of plants affected by this disease.

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SOME TESTS OF VARIETAL SUSCEPTIBILITY TO A COMBINATION OF ROOT-KNOT NEMATODE AND COTTON WILT[1]

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(Accepted for publication June 20, 1939)

The root-knot nematode is becoming increasingly important as a factor in cotton production, especially where it occurs in a soil infested with the cotton-wilt organism, *Fusarium vasinfectum* Atk. Under this combination the resistant qualities of even the most resistant varieties of cotton frequently are lost and heavy loss of plants results. This loss usually is attributed to wilt, and the wilt resistance of a variety is frequently unjustly discredited. In 1938 a test was begun at the Main Experiment Station, State College, Mississippi, to test the responses of a number of varieties and strains of cotton on land known to be heavily infested with both the root-knot nematode, *Heterodera marioni* (Cornu), and the cotton-wilt organism.

METHODS, MATERIALS, AND PROCEDURE

Seventeen varieties of upland cottons and 14 varieties and strains of foreign, exotic, and hybrid cottons were used. The seed of Tuxtlal, Hopi, Kekchi, Sakel, Pima, Acala × Hopi, and Sakel × Pima hybrids, as well as a selfed line of Missdel No. 4, was secured from the Division of Cotton and Other Fiber Crops and Diseases at Sacaton, Arizona. Seed of Sea Island 13B3 and Sea Island (Andrews) was secured from J. W. Neely, Cotton Breeder, Division of Cotton and Other Fiber Crops and Diseases, Stoneville, Mississippi.

One-row plots, 25 feet in length, were planted by hand, 5 seeds per hill, with the hills 6 inches apart, and covered to a uniform depth. The plots were replicated 8 times and thoroughly randomized. Plots were planted April 30, 1938.

Observations were made throughout the season. When the plants died they were removed and records were made as to the presence or absence of root-knots and internal discoloration caused by wilt. At the end of the season all plants were dug carefully and the roots examined. All showing knots were recorded as infested with nematodes. The plants were split and examined for wilt discoloration at the same time. Yield records were taken, though it was realized that the rows were too short for yield to have much significance.

Summarized results are presented in two tables. Table 1 represents the 17 commercial varieties of upland cotton. The entire number of varieties included was replicated 8 times. Table 2 represents the other 14 varieties and strains, which are presented separately for the reason that sufficient seed of certain ones was not available for the entire 8 replications. Pima, Sakel × Pima, and the two Sea Island strains were both replicated 8 times, while among the other 10 the number of replications varied from 3 to 5. Members of both tables, however, were planted in the same plot and thoroughly randomized. In order that there would be no missing rows in the plot, varieties and strains of which no seed was available for the later replications were replaced by Half & Half, but results from these extra rows of that variety are not included in the results presented.

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[1] Contribution from the Department of Plant Pathology, Mississippi Agricultural Experiment Station, State College, Mississippi. Published with the approval of the Acting Director, Mississippi Agricultural Experiment Station. Paper No. 18. New Series, May 29, 1939.

RESULTS

Examination of Table No. 1 reveals that the 17 varieties fall somewhat naturally into 3 groups on the basis of wilt infection. The group of varieties showing less than 25 per cent wilt at the end of the season included Clevewilt 6, Cook 144–68, Cook 307, Dixie Triumph 55–85, Toole (Perry), Sykes W. R., Dixie 14–5, and Dixie Triumph 12. The last one is included in this group, though it did show slightly more than 25 per cent wilt, because in all

TABLE 1.—*Relation of upland varieties of cotton to a combination of root-knot nematodes and wilt*

Variety	Average wilted	Average dead	Average wilted plus dead	Average nematode	Average yield seed cotton per acre
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Lb.*
Group I (Resistant)					
Clevewilt 6	13.92	4.59	17.07	45.63	1550.0
Cook 144–68	15.04	7.00	21.26	63.45	1237.5
Cook 307	15.07	17.61	26.83	60.00	1337.5
Dixie Triumph 55–85	15.56	13.12	25.01	54.14	1550.0
Toole (Perry)	20.53	8.71	27.36	58.15	1275.0
Sykes W. R.	20.96	15.16	30.66	47.87	1362.5
Dixie 14–5	22.15	8.38	27.74	59.50	1512.5
Dixie Triumph 12	25.05	2.75	24.79	70.91	1600.0
Average	18.53	9.66	25.09	57.49	1428.12
Group II (Intermediate)					
Rowden 2088	26.16	7.92	32.31	73.71	1225.0
Missdel W. R.	28.60	30.99	50.15	64.72	962.5
D & PL 11 A	39.59	14.42	47.83	58.07	987.0
Miller 610	42.48	18.50	50.62	74.76	1187.5
Carolina-del No. 2	46.26	12.56	53.35	63.61	912.5
Average	36.61	16.88	46.85	66.97	1054.90
Group III (Susceptible)					
Coker 100	68.75	41.03	81.01	67.74	812.5
Washington	70.44	29.54	78.40	75.25	612.5
Half & Half	72.52	48.44	85.58	53.83	400.0
Missdel No. 4	100.00	84.78	100.00	100.00	375.0
Average	79.92	50.95	86.25	74.13	550.0

other respects it belonged with the above group. This group contained the smallest numbers of plants showing death either during or at the end of the season and wilt at harvest time. Sykes W. R. showed a total of 30.66 per cent either dead or infected with wilt at harvest time. The group without exception contained the varieties showing the highest yield. The relationship to nematode infestation, however, is not so clear. Clevewilt 6, which showed the lowest wilt infection, also showed the lowest percentage infested with nematodes, 45.63 per cent. On the other hand, Dixie Triumph 12 showed an infestation of 70.91 per cent. The average percentage of plants infested with nematodes for the whole group, however, was lower than that of any other group namely, 57.49 per cent, as compared with 66.97 per cent for the next group, and 74.13 per cent for the third, or most susceptible group.

TABLE 2.—*Relation of exotic varieties, hybrids, and strains of cotton to a combination of root-knot nematodes and wilt*

Variety	Average wilted	Average dead	Average wilted and dead	Average nematode	Average seed cotton per acre
	Per cent	*Per cent*	*Per cent*	*Per cent*	*Lb.*
	Group I (Resistant)				
Sea Island 13B3	0	10.19	10.19	44.53	100.00
Sea Island (Andrews)	9.61	19.12	17.26	67.94	337.50
Hopi (Sacaton) 6 No. 2	9.83	31.57	39.47	98.07	187.50
Kekchi 7–8–5 No. 2	13.79	27.77	38.88	92.85	1375.00
Sakel 7–9 No. 2	14.47	18.29	31.70	89.55	125.00
Sakel × Pima (Sacaton)	14.60	27.82	36.08	82.40	153.75
Pima (Sacaton)	19.79	26.92	42.25	70.82	131.25
Acala × Hopi F_3–238 No. 311	23.52	17.50	40.00	96.96	1000.00
Average	13.20	22.39	31.98	80.64	423.7
	Group II (Susceptible)				
Tuxtlal 9–1 No. 5	59.09	26.92	76.92	100.00	1000.00
Acala × Hopi F_3–301 No. 11	64.00	54.34	89.13	100.00	625.00
Acala × Hopi F_3–197 No. 23	100.00	62.50	100.00	83.33	250.00
Acala × Hopi F_3–76 No. 9	100.00	77.77	100.00	100.00	625.00
Acala × Hopi F_3–365 No. 23	100.00	79.06	100.00	100.00	62.50
Hopi M 34–6–2 No. 6	100.00	100.00	100.00	100.00	0.00
Average	87.18	66.76	94.34	97.22	427.08

In the intermediate group, composed of Rowden 2088, Missdel W. R., D & PL 11 A, Miller 610, and Carolina-del No. 2, the variety Rowden 2088 approaches the varieties of group I. Reference to Table 1, however, will show that the group as a whole is relatively homogeneous and is clearly intermediate between group I and the most susceptible members located in group III. The last group was composed of the varieties Coker 100, with 68.75 per cent of wilt; Washington, 70.44 per cent wilt; Half & Half, 72.52 per cent, and Missdel No. 4, 100 per cent of which showed wilt symptoms, as well as root-knot from nematodes.

Although there was considerable variation among the varieties within a group, it will be noted that the yield per group decreased inversely as the percentage of wilt increased. Group I, with an average of 18.53 per cent wilt, yielded an average of 1428.12 pounds of seed cotton per acre; group II, average wilt, 36.61 per cent, gave an average yield of 1054.90 pounds per acre; and group III, wilt percentage 77.92 per cent, yielded 550 pounds. The relationship of nematode infestation among the varieties of a group was still more aberrant; but, taken by groups, an increase in nematode infestation was accompanied by an increased amount of wilt, an increased number of dead plants, and a decrease in yield.

The grouping of the remaining 14 varieties, strains, and hybrids is shown in Table No. 2. In these groups the relationships are not so clear-cut as in the case of the upland varieties. Group I, however, contains 8 members that are clearly more resistant to wilt than are the other 6. Sea Island 13B3 showed no trace of wilt at any time, while Sea Island (Andrews) showed a total of 9.61 per cent of the plants with typical internal discoloration. Of 5 third-generation Acala × Hopi hybrids, only one, Acala × Hopi F_3-238 No. 311, showed any resistance to wilt. This one showed only 23.52 per cent of the plants with wilt symptoms, and was included in group I. Only one variety, Sea Island 13B3, showed any resistance at all to nematode, with an infestation of 44.53 per cent. In this group the yield has little, if any, significance, since most members are represented by only 3 to 5 replications and since the rows were only 25 feet long. Most of the varieties did not fruit well at this location, though certain ones, such as Kekchi, Tuxtlal, and Acala × Hopi F_3-238 No. 311 gave yields comparable to the upland varieties.

Group II, comprising 6 members, showed very great susceptibility to both nematodes and wilt. One hundred per cent of the plants of Hopi M 34–6–2 No. 6, secured from Sacaton, Arizona, were dead at harvest time and all were infested with wilt and nematodes. No bolls opened on any plants of this strain of Hopi.

The 8 members making up group I of this collection of exotic varieties, strains, and hybrids showed a relatively higher wilt resistance than did the corresponding 8 comprising group I of the upland varieties; but, with the exception of Sea Island 13B3, they were more susceptible to the root-knot nematode. There was no intermediate group, and group II of the exotic strains and varieties was on the average more susceptible to both wilt and nematodes than even group III, the susceptible group, among the upland cottons.

SUMMARY

Seventeen varieties of upland cotton, and 14 varieties and strains of exotic and foreign cottons and their hybrids with upland cottons were grown on soil heavily infested with both the root-knot nematode, *Heterodera marioni* and the cotton wilt organism, *Fusarium vasinfectum.*

Of the upland cottons, 8 varieties, Clevewilt 6, Cook 144–68, Cook 307, Dixie Triumph 55–85, Toole (Perry), Sykes W. R., Dixie 14–5, and Dixie

Triumph 12, showed highest resistance to wilt and, as a group, relatively lower nematode infestation.

The varieties Rowden 2088, Missdel W. R., D & PL 11A, Miller 610, and Carolina-del No. 2 made up a group intermediate in resistance to wilt, and, as a group, were somewhat more heavily infested with nematodes.

Varieties Coker 100, Washington, Half & Half, and Missdel No. 4, were extremely susceptible to both wilt and nematodes when grown on soil infested with both.

Sea Island 13B3 showed no trace of wilt at any time, and also had the lowest percentage of plants infested with root knot among the entire 31 varieties represented in the test.

Another strain of Sea Island, designated as Andrews, showed 9.61 per cent wilt and a relatively high percentage of nematodes under the conditions of the test.

One strain of Hopi, designated as Hopi 6, No. 2, from Sacaton, showed only 9.73 per cent wilt, while another strain, Hopi M 34–6–2, from the same location, showed 100 per cent wilt. The first showed 98.07 per cent nematodes and the latter 100 per cent; in the latter case, however, all plants were dead before harvest.

Of 5 crosses between Acala and Hopi, 1 showed only 23.52 per cent wilt, another 64 per cent, and the remaining three 100 per cent. All were extremely susceptible to nematode.

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TOLUENE COMPOUNDS TO CONTROL PLANT DISEASE[1]

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(Accepted for publication June 24, 1939)

Chemotherapy may be a promising means of controlling certain plant diseases, although knowledge of its possibilities is at present very limited and the practicality of its use still somewhat doubtful. The difficulty lies in finding a chemical innocuous to the host plant but lethal for, or at least detrimental to, the parasite that is more or less protected within its host.

Chemical means of combatting cereal rusts have been tested experimentally in Europe. Applications of borax (sodium borate) to wheat plots for the prevention and control of leaf rust (*Puccinia triticina*) and stripe rust (*P. glumarum*) of wheat were recommended by Gigante[2] in 1935. Since that time Sempio,[3] Gassner and Hassebrauk,[4] and Hassebrauk[5] have tested many chemicals for their therapeutic value for several of the cereal rusts. The readily soluble picric acid proved to be better than borax; but, most promising of all the compounds tested, in Germany, were para-toluenesulfonylamide and ortho-toluenesulfonylamide; minute quantities of which injured the plants only slightly but hindered rust infection and altered the rust reactions of cereals from susceptibility towards resistance. The chemicals were particularly effective in inhibiting development of leaf rust of wheat. They were promising, too, with *Puccinia simplex* on barley, *P. dispersa* on rye, *P. glumarum* on wheat, and *P. graminis tritici* on wheat; but of doubtful value for *P. coronata* on oats.

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[1] Published with the approval of the Director as Paper No. 1712 in the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

[2] Gigante, R. Ricerche sopra l'influenza del boro sulla resistenza dele piante agli attacchi parassitari. Boll. R. Staz. Patol. Veg. Roma (n.s.) 15: 471–483. 1935.

[3] Sempio, C. Influenza di varie sostanze sul parassitamento: ruggine del fagiolo, ruggine el mal bianco del frumento. Rev. Patol. Veg. 26: 201–278. 1936.

[4] Gassner G., and K. Hassebrauk. Untersuchungen zur Frage der Getreiderostbekämpfung mit chemischen Mitteln. Phytopath. Zeitschr. 9: 427–454. 1936.

[5] Hassebrauk, K. Weitere Untersuchungen über Getreiderostbekämpfung mit chemischen Mitteln. Phytopath. Zeitschr. 11: 14–46. 1938.

We have tested the efficacy of the recommended chemicals for controlling stem rust of wheat, using the widespread race 56 of *Puccinia graminis tritici* for our experiments.* Seedlings of the susceptible vulgare wheats, Marquis and Ceres, and of the resistant durum variety, Mindum, grown in 4-inch clay pots in the greenhouse, were treated with borax, picric acid, para-toluenesulfonylamide, or ortho-toluenesulfonylamide 6 to 7 days after planting and 2 to 3 days before inoculation with rust urediospores. Chemicals were mixed with quartz sand and strewn over the soil surface so that they would be dissolved and become available to the plants as the pots were watered. Their effect on rust infection and the extent of seedling injury resulting from their use are given in table 1.

The percentages of plants rusted were consistently high (90 per cent) in the check series. They were lower in all the series receiving chemicals, being reduced to approximately 20 per cent in the two susceptible wheats by the two toluene compounds. In addition, there were generally fewer infection centers, in all three wheat varieties, when the chemical treatments were used.

The reaction type was not altered by treatment with borax or picric acid. Normal uredia, exactly like those on corresponding varieties in the check series, developed on plants treated with these chemicals in spite of the reductions in prevalence and severity of rust. The para- and ortho-toluenesulfonylamide, however, lowered the reaction type in addition to reducing the number of uredia. Uredia on Marquis and Ceres treated with the toluene compounds were extremely small and sporulation was very limited. The tissues surrounding the pustules remained green, with no indication of chlorosis, necrosis, or a hypersensitiveness that would throw the reaction into type 2 or 1. We were loathe to classify the reaction as type 3, for the rust was definitely subnormal and underdeveloped, but we recorded it as a 3≡ reaction.

All chemicals injured the seedlings to some degree. In the first experiment an application of borax at the rate of 8 g. per sq. m. of soil surface burned the wheats so severely that rust did not infect them. Even with reduction in the amount of borax to 0.64 g. per sq. m., the injury was sufficiently severe so that a considerable portion of the assimilating foliage was destroyed and recovery or regaining of normal vigor was improbable. With

TABLE 1.—*The effect of chemicals on wheat seedlings and on their infection by Puccinia graminis tritici 56*

Rust infection and injury on 3 varieties of wheat	Chemical and rate of application per sq. m. soil surface				
	Check: no treatment	Borax (sodium borate)[a] 0.64 g.	Picric acid 12 g.	Para-toluenesulfonylamide 1 g.	Ortho-toluenesulfonylamide[a] 0.8 g.
MARQUIS					
No. plants rusted	58	7	29	8	4
Total no. plants	63	20	38	44	21
Rust reaction type[b]	4	4	4	3≡	3≡
Foliage injury	None	Moderate burning on basal leaves	Foliage yellow-green; tip burning on basal leaves	Slight tip burning	Slight tip burning
CERES					
No. plants rusted	58	9	28	8	4
Total no. plants	64	22	40	37	19
Rust reaction type	4–	4–	4–	3≡	3≡
Foliage injury	None	Moderate burning on basal leaves	Foliage yellow-green; tip burning on basal leaves	Slight tip burning	Slight tip burning
MINDUM					
No. plants rusted	58	0	10	6	0
Total no. plants	62	22	41	42	20
Rust reaction type	0; to 1=		1=	0; to 1≡	
Foliage injury	None	Moderate to severe burning on basal leaves	Foliage yellow-green; mod. to severe burning on basal leaves	Tip burning	Tip burning

[a] In one experiment foliage injury was so severe and extensive after treatment with 8 g. of borax per sq. m. that the plants succumbed and no rust developed. In one experiment minute applications of ortho-toluenesulfonylamide, at the rate of 0.2 g. per sq. m., failed to reduce or affect in any way infection by stem rust.

[b] The rust reaction type was determined according to the standards established by Stakman and Levine in The determination of biologic forms of *Puccinia graminis* on Triticum spp., Minn. Agr. Exp. Stat. Tech. Bull. 8, 1922. The scale is from 0, which indicates immunity, to 4, which indicates extreme susceptibility.

picric acid treatment the plants became yellow from the quantities of the chemical carried in the transpiration stream. The burning was moderate, not so severe as with borax, but yet more pronounced than with the toluene compounds. The general vigor of the yellowed plants was below that of check plants. Injury from the two toluene compounds was confined to slight but definite tip burning on the basal leaves, from which the plants recovered so rapidly that their general vigor was comparable with that of check plants and occasionally seemed even to exceed it. Soil type may influence the effect of toluene compounds, because the reduction in rust was somewhat more marked on a sandy soil than on a loam.

Little yet is known regarding the recovery from injury, the limits of tolerance, and the dissipation of therapeutic values; but we have been able to reduce stem-rust infection on wheat by chemical means, and our own greenhouse experiments corroborate the results of Hassebrauk.

The toluenesulfonylamide compounds were so remarkably effective in these greenhouse experiments that, in spite of their high cost, extensive tests of their possible value as therapeutic agents for cereal rusts, for diseases of ornamental plants and shrubs, and for other plant diseases is strongly recommended. It may not be easy, or even possible, to devise a practical and inexpensive treatment for field crops; but, for some of the ornamental plants produced in greenhouses or nurseries and with relatively high individual plant values, the toluene compounds may be useful and their cost may not be prohibitive in controlling plant disease.

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IMMUNITY OF A STAMINATE CLONE OF RIBES ALPINUM FROM CRONARTIUM RIBICOLA

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(Accepted for publication June 20, 1939)

INTRODUCTION

The blister-rust susceptibility of the ornamental alpine currant, *Ribes alpinum* L. of the Old World, which is becoming locally popular in landscape gardening in the Middle West, has been variously stated. Spaulding (7), Tubeuf (8), Kouba,[1,2] and the writer (5), working with a clone studied by Spaulding, reported this dioecious species as immune from, or highly resistant to, blister rust (*Cronartium ribicola* Fischer). On the other hand, Schellenberg (6) in Switzerland listed the currant as highly susceptible. Clinton and McCormick (2) reported pistillate plants as susceptible to *C. ribicola* and staminate plants as immune from not only white-pine blister rust, but also piñon blister rust, *C. occidentale* Hedgc., Bethel, and Hunt.

The apparently conflicting reports concerning the susceptibility of *Ribes alpinum* prompted the writer to investigate the species further to find out whether there was really a difference in susceptibility between staminate and pistillate plants of the species. Between 1930 (5) and the time he performed the experiments on *R. alpinum* described in this paper, the writer lost sight of the fact that Clinton and McCormick (2) already had discovered a difference in rust-susceptibility between the two sexes. The importance of this discovery, which was not emphasized at the time it was made, has remained unrecognized by blister-rust investigators up to the present time.

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[1] T. F. Kouba. Experiments to determine the susceptibility of *Ribes alpinum* Linn., in, U. S. Dept. Agr. Plant Indus., Blister Rust News 16: 229. 1932 (Mimeographed).

[2] T. F. Kouba. Susceptibility of the alpine currant to white pine blister rust, in, *Ibid.* 17: 165. 1933 (Mimeographed).

INOCULATION RESULTS

For the experiments on *Ribes alpinum* described in this paper, the writer had available an excellent specimen of the staminate plant growing in the Marsh Botanical Garden, Yale University, New Haven, Connecticut. The authenticity of this material was corroborated by Alfred Rehder of the Arnold Arboretum, Harvard University. Artificial inoculation tests were performed in 1937 and 1938 with material from this single clone. On May 19, 1937, a cloudy day when light rain was falling, the staminate clone was heavily inoculated with the viable, freshly collected aeciospore inoculum of *Cronartium ribicola* taken in Connecticut. The test was repeated on June 4 with viable aeciospores gathered in Maine and subsequently stored in an ice box. This plant, together with known-susceptible *Ribes*, was covered at the time of inoculation with an iceless-refrigerator type of moist chamber (3). A total of 450 inoculated leaves in both tests on the staminate clone of *R. alpinum* proved to be immune from blister rust, whereas the susceptible *Ribes* "checks" were so heavily infected that they became defoliated.

Since only staminate material of *Ribes alpinum* was available at New Haven, cuttings taken from a single pistillate clone growing in the *Ribes* garden at the Arnold Arboretum were obtained in 1937 for parallel testing. Plants of that sex, which were the only ones growing at this time at the Arboretum, had been reported in October, 1931, as showing a natural light infection with *Cronartium ribicola* by L. W. Hodgkins,[3] Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, U. S. Department of Agriculture. Cuttings of this material, standing in a beaker of water in a pathological greenhouse, were dusted on July 28 with aeciospore inoculum from Maine that had been stored in an icebox since its collection in May. A total of 60 fully mature leaves of the cuttings were inoculated. These showed a scant (slight) degree of infection (5, p. 109) of rust on 18 leaves. As previously reported the relative abundance of uredia produced on the leaves of cuttings of susceptible *Ribes* was not so great as that produced subsequently on inoculated leaves of potted plants (4, p. 144).

In 1938, inoculation tests were repeated on potted specimens of both sexes of *Ribes alpinum* in the greenhouse. This stock was propagated from cuttings taken, respectively, from the staminate clone growing in the Marsh Botanical Garden and from the pistillate clone growing at the Arnold Arboretum. It is here interesting to note that plants of boths sexes grew readily from cuttings that had produced previously an abundance of adventitious roots during the summer while standing in ordinary tap water in the greenhouse. On May 14, 1938, 4 of the potted staminate plants (185 leaves) together with 4 pistillate plants (279 leaves) were inoculated with viable aeciospore inoculum freshly collected in Connecticut. Two additional potted staminate plants (108 leaves), together with 1 potted pistillate plant (63 leaves), were tested similarly on May 27. In this particular test the checks consisted of 2 large potted plants of the susceptible red garden currant, American Red Dutch [*Ribes sativum* (Rchb.) Syme hybrid]. Additional outdoor inoculations were made on May 27 on the staminate plant of *Ribes alpinum* in the Marsh Botanical Garden with freshly collected viable aeciospores from Maine. A total of 2011 leaves in all stages of development were tested.

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[3] Filler, E. C. Blister rust on Ribes at the Arnold Arboretum, in, U. S. Dept. of Agr. Plant Indus., Blister Rust News 15: 243. 1931 (Mimeographed).

Final observations were made on August 17 on the inoculated plants both inside and outside the greenhouse. None of the 2304 inoculated leaves of the staminate material of *Ribes alpinum* showed blister rust infection of any sort (Fig. 1, A). Of the 342 leaves of the pistillate clone inoculated in the greenhouse, 173 leaves were scantily to heavily infected. The average degree of infection for uredia was rated as moderate and these were associated with a scant telia production and necrotic leaf spots. Considerable defoliation occurred on the infected pistillate plant, shown in figure 1, B.

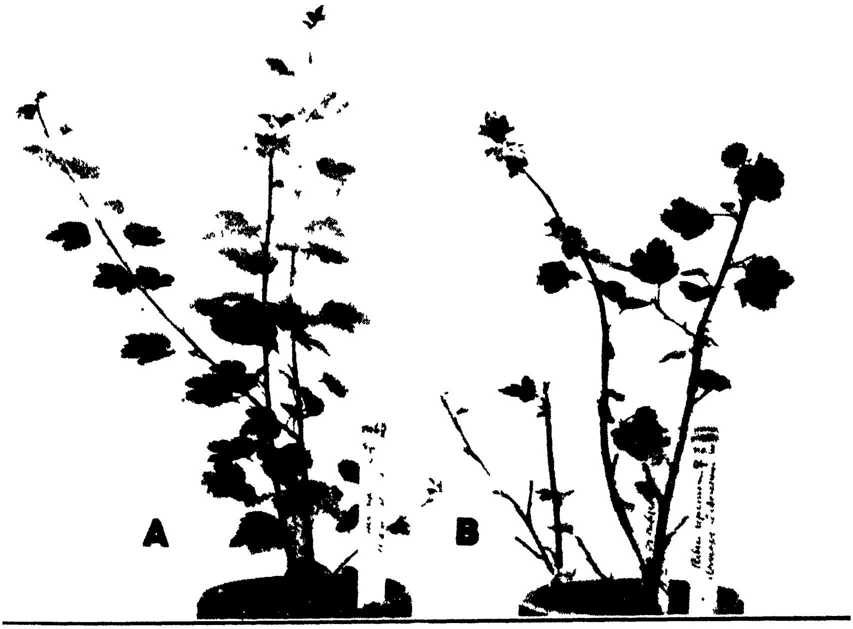

FIG. 1. Staminate and pistillate plants of *Ribes alpinum* inoculated with aeciospores of *Cronartium ribicola*, May 14, 1938. A. Immune staminate plant propagated from specimen growing in the Marsh Botanical Garden, Yale University; B. Susceptible pistillate plant propagated from a specimen in the Arnold Arboretum, Harvard University. The rust caused considerable defoliation. Photographed on July 9, 1938 by G. K. Goodling.

In every experiment known-susceptible *Ribes* inoculated to establish the viability of the inoculum and the suitability of the environmental conditions became infected. In the 1938 tests it is important to comment upon the fact that spores of *Cronartium ribicola*, formed on susceptible *Ribes*, were floating about in the pathological greenhouse during the summer and autumn. The presence of free spores was indicated by natural infection of blister rust on leaves of potted plants of the very susceptible European black currant, *Ribes nigrum* L., which showed abundant viable telia late in the autumn. On the other hand, plants propagated from the staminate clone of *R. alpinum* remained free from infection throughout the growing season.

DISCUSSION

Type of Rust Resistance Shown by Staminate Plants of *Ribes alpinum*

Necrotic flecks associated with blister rust infection, such as those described by the writer (4) and Anderson (1) as forming on the leaves of the fruitful, rust-immune Viking currant (Syn. Rød Hollandsk Druerips, *Ribes rubrum* L. × *R. petraeum* Wulf.) from Norway, were lacking on the leaves of the staminate plant of *R. alpinum*. Moreover, necrotic lesions similar to those described and figured by Spaulding (7, Pl. 5) for blister rust infection on the leaves of the cultivated red currant varieties [*R. sativum* (*vulgare*) hybrids], were also lacking on plants of this sex. These necrotic spots, associated with a scant (slight) degree of telia production, were observed, however, on leaves of the susceptible American Red Dutch checks (5, p. 116). When these red currants were examined on August 17 their leaves had hardened. Moreover, in the greenhouse (5, p. 108), they had not formed new leaves during the summer and those that had been produced were becoming yellowed and were dropping from the plants.

Results of Other Workers with *Ribes alpinum*

When Spaulding (7) reported his negative results on *Ribes alpinum*, he did not record whether they were obtained on staminate or pistillate clones. Although he procured cuttings of both sexes from the Arnold Arboretum in 1915, a record was not kept of the sexes of the young plants that were successfully propagated. It is possible that Spaulding's negative results were obtained on a staminate clone, for Clinton and McCormick (2), who also obtained *R. alpinum* material of both sexes from the Arboretum 2 years later, demonstrated that their staminate plants were the only ones immune from blister rust. Spaulding's results were corroborated in part by those of the writer (5), who, in November 1921, took cuttings of one of the plants of *R. alpinum* used by Spaulding and found that the young plants propagated from this single clone were immune. Since the time these tests were made, the experimental currants at Washington, D. C., have been destroyed, so that it is no longer possible to determine the sex of the immune specimen used by Spaulding and later by the writer in 1921.

In 1917 to 1919, Clinton and McCormick (2) using both aeciospores and urediospores of *Cronartium ribicola* as inoculum, performed 11 tests on pistillate plants, 5 of which were made on plants in pots and 6 on leaves in Petri dishes. Failure occurred in two of these tests; infection ranged from poor (P) to excellent (E), and their average rating for degree of infection was fair minus (F –). We do not know whether their plant material of each sex came from one or more clones, for records were not kept to clear up this point.[4] Possibly the susceptible, pistillate plant material from the Arnold Arboretum, tested by the writer in 1937 and 1938, may have come from a clone investigated by Clinton and McCormick.

Parellel tests with staminate material consisting of inoculations on leaves of *Ribes alpinum* in Petri dishes and on potted plants were carried on also by the Connecticut workers (2). Negative results were obtained in 13 tests. In all probability our staminate material did not come from the same source whence Clinton and McCormick obtained their specimens. The writer was unable to procure exact information on the origin of the staminate clone he investigated other than that it probably was obtained from some nursery.

Additional weight to the writer's and Clinton and McCormick's results with immune staminate plants is given by the results of Kouba.[5] He found that non-fruiting and probably staminate material of *Ribes alpinum* procured from a nursery in Iowa (written communication, Oct. 31, 1938) was immune from blister rust. Kouba's plants were growing for a number of years near infected northern white pine (*Pinus strobus* L.) and associated with susceptible *Ribes* that became infected each year under natural conditions.

All of these investigations collectively give impetus for further study of other staminate clones of *Ribes alpinum.* Additional work also should be performed with a number of clones of pistillate material to test further their susceptibility to blister rust. Inasmuch as the grower is interested primarily in the vegetative growth of the species, the immunity of the staminate plant becomes a matter of importance.

SUMMARY

A clone each of the staminate and pistillate plants of the alpine currant, *Ribes alpinum,* was inoculated with *Cronartium ribicola* in 1937 and 1938. Aeciospores collected in Connecticut and Maine were used as inoculum.

A total of 2754 leaves of the staminate clone of *Ribes alpinum* were inoculated, all of which proved to be immune. The pistillate clone, together with known-susceptible *Ribes* "checks," inoculated to establish the viability of the inoculum and the suitability of the environmental conditions, became infected.

The reaction of leaves of the immune staminate plant to blister rust infection is compared with that of (1) leaves of the immune Viking red currant and (2) leaves of the susceptible American Red Dutch currant checks.

A discussion also is given of the results of other workers who have experimented with the alpine currant. The results stated in this paper cor-

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[4] Information given the writer through the courtesy of Dr. McCormick.

[5] See footnotes 1 and 2.

roborate the earlier findings of Clinton and McCormick, who discovered but did not emphasize a difference in rust-susceptibility between the two sexes of this European currant used extensively in the Lake States Region for ornamental planting.

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EFFECT OF TYPE AND PERIOD OF STORAGE ON COTTON SEED AFTER TREATMENT WITH ORGANIC MERCURY DUSTS[1]

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Cottonseed producers and cotton farmers frequently have asked how long before planting could they treat their cottonseed with organic mercury dusts, such as Ceresan and New Improved Ceresan, without injury to the seed and with beneficial results in emergence and yield, and, also, what effect would different types of storage after treatment with such dusts have on the treated seed. Up to the present time there has been little, if any, published information based on actual tests along these lines. A test was designed and begun in 1936 to answer, in some measure, these questions.

MATERIALS AND METHODS

Cottonseed of the 1936 crop, variety D & PL 11 A, sometimes called Deltapine A, secured direct from the producers, the D & PL Co., Scott, Mississippi, was used. The harvest season in Mississippi in 1936 was very dry. As a consequence, the seed was excellent, relatively free from seed-borne disease, and with a high percentage of germination. A sufficient amount of seed was secured to carry the test through two planting seasons.

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[1] Contribution from the Department of Plant Pathology, Mississippi Agricultural Experiment Station, State College, Mississippi. Published with the approval of the Acting Director, Mississippi Agricultural Experiment Station. Paper No. 19. New Series, May 29, 1939.

Two types of storage were used. One portion of each treated and nontreated lot was placed in the laboratory where the temperature was uniformly high and the air-moisture low. The other portion was stored in a ventilated, galvanized-iron, rat-proof corncrib, located in the open field. In this the seed was subjected to all changes of temperature and moisture characteristic of the crib throughout the storage period, though, of course, protected from free water in the form of rain or snow. The seedlots were placed in small cloth sacks but were stacked up so as to approximate somewhat the conditions of ordinary commercial storage. The seed, unused for planting in 1937, both treated and nontreated, was kept under these respective storage conditions until used in the spring of 1938.

On November 15, 1936, one lot of seed was dusted with 2 per cent Ceresan at the rate of 3 oz. per bu. of seed, another was dusted with New Improved Ceresan at the rate of 2 oz. per bu., and a third lot was left nontreated. A portion of each lot was placed in dry storage in the laboratory and a similar portion was placed in outside storage, as described above. On about the 15th day of each month thereafter, up to planting time, April 11, 1937, similar lots were treated and placed in storage, both inside and outside. In the interval between securing the seed from the grower and treatment, all seed was maintained in dry storage in the laboratory. On date of planting there were available, therefore, lots nontreated, treated with 2 per cent Ceresan, and treated with New Improved Ceresan, which had been in storage under the respective storage conditions for periods of 1, 2, 3, 4, and 5 months, respectively. Other lots that had been stored in the laboratory without treatment were treated at planting time to serve as checks on the effect of type and period of storage after treatment.

The seed was planted by hand at a uniform depth of approximately 1 in. in rows 40 in. apart and 50 ft. long. Each row contained 100 hills, 6 in. apart and containing 5 seeds each. All were fertilized uniformly with 600 lb. per acre of a 6–8–4 fertilizer applied in the row below the seed about 2 weeks prior to planting. Each lot was replicated 8 times and thoroughly randomized so that the data could be analyzed statistically.

Emergence counts were made after all plants were up and the exact percentages of emergence calculated. Yield data, taken at harvest time, were expressed as pounds of seed cotton per acre.

RESULTS IN 1937

Under the conditions of the test, the type of storage used had no significant effect on the results. For all lots stored inside, regardless of treatment or period of storage after treatment, the average percentage emergence was 64.39, while the average yield was 1605.94 lb. of seed cotton per acre. For the lots stored outside, the average percentage emergence was 64.50 and the average yield 1671.32 lb. per acre. These differences were not significant statistically.

There was an average decrease in yield of 3.58 per cent for all seedlots stored after treatment as compared with the yield from seed treated immediately before planting. This decrease, however, proved statistically insignificant.

The data showed also that, irrespective of the organic mercury dust used, the period of storage after treatment up to 5 months, the maximum for this test, was of no consequence. The largest difference in average percentage of emergence for any storage period in the lot stored inside, as compared with the unstored lot, was only 3.45 per cent, while a difference of 5.45 per cent was required for significance. For the lot stored outside, the largest average difference was only 2.79 per cent, while that required for significance was 5.85 per cent. Statistical analysis of the data shows also that in yield, as well as in emergence, the effects of storage after treatment with either dust, up to a 5-month period, are unimportant.

The effects of the treatments themselves, however, irrespective of type or period of storage, were very marked. The average increase in emergence over the nontreated check for the lots treated with 2 per cent Ceresan and New Improved Ceresan, respectively, were 25.1 per cent and 24.6 per cent. The average increase in yield as a result of seed treatment was 24.6 per cent for the 2 per cent Ceresan and 26.4 per cent for the New Improved Ceresan. The increase necessary for significance at odds of 99 to 1 was only 8.62 per cent. Table 1 will show that increases in yield were in the main closely associated with increased emergence, though such was not always the case.

TABLE 1.—*Increase in emergence of seedlings and in yield per acre of seed cotton from seed treated with organic mercury dusts and stored after treatment for different periods of time and under different conditions of storage, then sown in 8 randomized replications, April 11, 1937*

Storage after treatment		Data from nontreated seed		Increase following treatment of seed with			
				Ceresan		New Imp. Ceresan	
Type	Period	Emergence	Yield	Emergence	Yield	Emergence	Yield
	Months	*Per cent*	*Pounds*	*Per cent*	*Per cent*	*Per cent*	*Per cent*
In laboratory	0	55.7	1460.9	25.5	26.7	16.9	23.0
	1	54.6	1382.8	26.9	20.1	16.4	31.1
	2	57.0	1507.8	17.2	18.0	18.7	20.2
	3	53.8	1539.4	31.2	28.3	19.3	31.7
	4	59.4	1414.1	22.2	30.5	16.4	25.4
	5	52.9	1414.1	38.4	36.8	24.4	27.1
	Average	55.6	1453.2	26.9	26.7	18.7	26.4
In outdoor crib	0	54.5	1487.5	27.9	23.1	26.1	21.5
	1	58.8	1476.6	16.9	18.6	22.8	26.5
	2	58.4	1367.2	23.3	14.6	32.0	33.1
	3	52.7	1304.7	27.7	30.0	38.3	29.9
	4	56.2	1437.5	29.3	25.6	28.3	16.8
	5	55.8	1373.4	14.5	22.0	36.0	30.8
	Average	56.1	1407.8	23.3	22.0	30.6	26.4
	Average of both	55.9	1430.5	25.1	24.5	24.6	26.4

RESULTS IN 1938

On April 15, 1938, seedlots that had been maintained constantly in storage of the two types previously described were planted in the same manner as in the preceding year. Of the nontreated seed, which also had passed through the storage period, one portion was planted without treatment as a check against treatment and the two others were treated with the respective dusts as a check against the effects of storage after treatment. This test, again, was replicated 8 times and emergence counts and yield records were taken as in the previous year. Two of the lots had, therefore, been in storage for a period of 17 months after treatment with 2 per cent Ceresan and New Improved Ceresan, respectively (Table 2).

TABLE 2.—*Increase in percentage emergence of seedlings and in yield per acre of seed cotton from seed treated with organic mercury dusts either 17 months or immediately before planting, portions of both lots of seed having been stored under different conditions for 17 months prior to planting on April 15, 1938, in 8 randomized replications*

Storage after treatment		Treatment applied to seed	Emergence	Yield per acre	Increase, caused by treatment, in	
Type	Period				Emergence	Yield
	Months		*Per cent*	*Pounds*	*Per cent*	*Per cent*
In laboratory		None	52.9	912.5		
	0	Ceresan	63.0	1144.4	19.2	25.4
	0	N. Imp. Cer.	68.2	1185.9	29.0	30.0
	17	Ceresan	66.5	1144.9	25.8	25.5
	17	N. Imp. Cer.	76.6	1269.5	44.9	39.1
In outdoor crib		None	56.9	928.9		
	0	Ceresan	67.9	1075.8	19.3	15.8
	0	N. Imp. Cer.	73.7	1168.4	29.5	25.8
	17	Ceresan	69.7	1195.7	22.4	28.7
	17	N. Imp. Cer.	75.6	1231.6	32.8	32.6

Again we find that the type of storage, either inside or outside, even over a period of 17 months, was not important. The average difference in emergence between the two types of storage was only 3.4 per cent, while at odds of 99 to 1 a difference of 4.28 per cent was required for significance. The difference in yield between the two types of storage, irrespective of other factors, was only 11.4 lb. of seed cotton per acre.

In the 1938 test, only two periods of storage were considered, that is, 17 months as compared with no storage after treatment. Reference to table 2 will show that, instead of injury resulting from this relatively prolonged storage period after treatment, the opposite was the case, and increases in emergence and yield resulted as compared with the lots treated at planting time. Analysis of the data with regard to emergence reveals, however, that the increases were not large enough for significance, except in the case of New Improved Ceresan, which did give a significant increase over 2 per cent Ceresan after a storage period of 17 months; whereas the difference between the two was not significant when the seed was planted immediately after treatment. With regard to yield, as well, the differences were not large enough to be significant when the data were analyzed in accordance with statistical methods.[2]

When, however, we consider the effects of the treatments themselves, whether applied immediately or 17 months before planting, we find remarkable increases both in emergence and yield. Ceresan increased emergence over the nontreated seed by 19.3 per cent when used at planting time and 24.1 per cent when applied 17 months prior to planting. The corresponding increases for New Improved Ceresan were 29.3 and 38.9 per cent, respectively. Ceresan brought about an increase of 20.6 per cent in yield when applied at planting time and 27.9 per cent when applied 17 months before planting. For New Improved Ceresan the corresponding figures were 27.1 per cent and 35.8 per cent, respectively.

These results closely confirm those secured in the preceding year in showing that neither the type of storage within the limitations of the test nor the period of storage after treatment, even up to 17 months, is apt to cause injury from treatment, of cottonseed with either 2 per cent Ceresan or New Improved Ceresan. They also show that both materials, when applied to cottonseed, are highly effective in bringing about increases in emergence and yield, even though the seed be relatively free from disease and of high quality in other respects.

DISCUSSION AND CONCLUSIONS

The high yields resulting from treatment of the seed with organic mercury dusts in this test are rather difficult to explain, especially when one considers that the nontreated seeds, themselves, produced a uniform and rather satisfactory stand. It would seem probable that some factor other than the control of seed-borne disease must have entered into the effect.

A rather extensive cotton-seedling-disease survey was conducted in North Carolina, South Carolina, Georgia, Alabama, Tennessee, Arkansas, Louisiana, Texas, and Mississippi during the spring months of 1938, by the U. S. Plant Disease Survey in cooperation with the Division of Cotton and Other Fiber Crops and Diseases and the Mississippi Agricultural Experiment Station. This survey showed that cotton seedlings collected in Mississippi had a higher percentage of infection with "sore-shin" and damping-off, caused by *Rhizoctonia solani*, than did those of any other of the States surveyed. Taking this fact into consideration it would seem probable that at least part of the yield increase resulted from control by the dusts of post-emergence damping-off and infection by soil-borne organisms.

On the other hand, if such were the case, one would expect Ceresan to be more effective than the New Improved Ceresan in combating such organisms, especially after so long a storage period, since it is much less volatile and would be expected to be less likely to have become dissipated during that period of time. The facts were that New Improved Ceresan, after storage, produced greater increases in both emergence and yield than did 2 per cent Ceresan.

[2] Love, H. H. Applications of Statistical Methods to Agricultural Research (1938). The Commercial Press, Changsha, China.

In any event, the test showed clearly the beneficial effects of seed treatment, even on seed that was above the average of that generally used, both in respect to germination and freedom from seed-borne diseases. It also showed that seed could be safely treated at any time between harvest and subsequent planting date, and that, if necessary, any unused treated seed could safely be held over until the second planting season with entirely satisfactory results.

MISSISSIPPI STATE AGRICULTURAL EXPERIMENT STATION,
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CEDAR BLIGHT ON WILDING AND FOREST TREE NURSERY STOCK

WILLIAM C. DAVIS AND DENNIS H. LATHAM

(Accepted for publication June 6, 1939)

Increased use of red cedar (*Juniperus virginiana* L.) in erosion control and windbreak plantings during the last few years has directed more attention than in the past to its juvenile diseases. Probably the most severe disease losses of red cedar nursery stock east of 105° longitude in the United States are caused by *Phomopsis juniperovora* Hahn. Cedar blight was reported in detail by Hahn *et al.*[1] in 1917, and the causal fungus was described as a new species in 1920.[2] In 1937 and 1938, the writers observed this fungus killing wilding red cedar seedlings in natural stands in Virginia, North Carolina, and Tennessee.

Published reports of the occurrence of *Phomopsis juniperovora* on native cedars as a source of infection in nurseries are lacking except for one instance cited by Hahn *et al.*[1] In 1900 G. G.Hedgcock made this collection on small branchlets of native red cedar in eastern Nebraska, where nursery seedlings of the Platte River type of juniper have been seriously affected by the disease.

Despite the paucity of data relative to the infection of native junipers by *Phomopsis juniperovora,* it has been assumed that such trees were infected and that they served as the source of nursery infection. In the fall of 1937 and again in the spring and fall of 1938 the writers isolated the associated fungus from 3- to 20-year-old diseased natural reproduction of red cedar in North Carolina, and in February, 1937, and September, 1938, from 2- to 8-year-old diseased native red cedar in Tennessee. The symptoms of the disease on wilding red cedar are the same as those illustrated for nursery stock by Hahn *et al.*[1] On older native red cedar, however, but few cankers have been observed by the writers, the symptoms most frequently found being dead shoots on the leaves of which the parasite was found fruiting. One of the isolates was identified by Hahn as *P. juniperovora.* Cultural characteristics of the other isolates, when grown on corn-meal agar, and measurements of the α and β spores of these isolates agreed with the description of *P. juniperovora* Hahn and appeared identical with the transfer Hahn identified. Most of the isolations made in North Carolina were from wildings or trees in the immediate vicinity of nurseries, but most of those made in Tennessee were 8 or more miles from nursery sites. At an Alabama and a North Carolina nursery in the vicinity of which there were no native red cedar trees, the disease was not observed in 1938. Further observations are desirable relative to a possible correlation between severity of the disease in nurseries and the presence of *P. juniperovora* on native trees in their immediate vicinity.

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[1] Hahn, Glenn G., Carl Hartley, and Roy G. Pierce. A nursery blight of cedars. Jour. Agr. Res. [U.S.] 10: 533–540. 1917.

[2] Hahn, Glenn G. *Phomopsis juniperovora*, a new species causing blight of nursery cedars. Phytopath. 10: 249–253. 1920.

In most Federal and State nurseries in the Southeast and Central States region, cedar blight caused severe losses in 1937 and 1938. *Phomopsis juniperovora* was isolated by the authors from diseased red cedar nursery stock in 1938 in North Carolina, Virginia, Tennessee, and Iowa.

The susceptibility of species of the Cupressaceae to cedar blight and control treatments has recently been summarized by Davis *et al.*[3] In control tests in 1938, in cooperation with Soil Conservation Service nurserymen, it was possible to observe the effect of fertilizer on the disease. Both ammonium sulphate and a 4–10–4 commercial fertilizer stimulated the early growth of 2-year-old transplanted red cedar in a North Carolina nursery. By fall, however, the losses caused by cedar blight were about twice as large on the fertilized as on the unfertilized plots, even where roguing and spraying were employed. Similar tests with ammonium sulphate at 2 Indiana nurseries also stimulated early growth, and more severe losses occurred on fertilized than on the nonfertilized plots.

Control practices of spraying with Bordeaux and roguing rigorously enough to produce blight-free stock at one nursery cost 40 cents per 1,000 seedlings.[4] At another they were so costly as to be considered prohibitive. At least after epidemics have gotten under way the practical advantages of roguing and spraying, as they have been practiced, are dubious; improved techniques are being tested.

CIVILIAN CONSERVATION CORPS IN COOPERATION WITH
DIVISION OF FOREST PATHOLOGY,
BUREAU OF PLANT INDUSTRY,
UNITED STATES DEPARTMENT OF AGRICULTURE.

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[3] Davis, William C., George Y. Young, Dennis H. Latham, and Carl Hartley. Diseases of conifers in forest nurseries. U. S. Dept. Agr., Bur. Pl. Ind. Mimeograph. 63 pp. 1938.

[4] Information from Bowen S. Crandall.

THE IMPORTANCE OF STANDARDIZED PROCEDURES IN DILUTING LIQUID LIME SULPHUR[1]

H. W. THURSTON, JR. AND DONALD E. H. FREAR

(Accepted for publication June 20, 1939)

In the use of any fungicide so complex and variable in its composition as liquid lime sulphur, it is difficult at best for the user to be sure of either the fungicidal value or "phytocidal" properties of recommended dilutions. It has been the custom in this country to consider either the specific gravity or Beaumé test of the concentrate as providing a reliable index to the amount of active fungicidal ingredients present. Since, however, the value of lime sulphur probably depends solely on the amount of calcium polysulphides present, while the specific-gravity reading is a function of the total solids in solution, it would seem more logical to base dilutions on the guaranteed polysulphide sulphur content, as is done in England, than upon a specific-gravity or Beaumé reading. Safro,[2] in 1913, was one of the first of many who have pointed out the inadequacy of the Beaumé test as a standard for dilution.

The writers have recently had occasion to analyze 30 samples of lime-sulphur concentrate, representing 8 different commercial brands, and 5 sources of home-boiled concentrate.[3]

These samples are believed to represent a fair cross section of the material available to Pennsylvania fruit growers, and, in all probability, of the country at large.

The analyses are presented in table 1, and reveal several facts of considerable practical importance. It will be noted that there is not a little variation in both the specific gravity and the polysulphide content, the former varying from 1.182 to 1.293 (22.33° to 32.86° Beaumé) and the latter from 10.90 per cent to 25.94 per cent. When these data were analyzed statistically there was found a correlation coefficient of 0.919 between specific gravity and polysulphide content, indicating that specific gravity is, after all, a fairly reliable index of fungicidal value.

Taking as an example the highest (sample 24) and the lowest (sample 2) figures for specific gravity, and assuming that both samples are diluted 1–75, the diluted spray made from sample 2 would contain 0.171 per cent polysulphide sulphur, while a similar dilution of sample 24 would contain 0.445 per cent; more than 2½ times as much. In other words, to get the same final concentration of the active ingredients in the diluted spray, the user would require more than 2½ times as much of the original lime-sulphur concentrate represented by sample 2 as would be used if sample 24 were diluted.

It should be pointed out, on the other hand, that if the procedure of

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[1] Authorized for publication on August 2, 1939 as paper No. 919 in the Journal Series of the Pennsylvania Agricultural Experiment Station.

[2] Safro, V. I., An investigation of lime sulphur injury. Oreg. Res. Bull. 2. 1913.

[3] Acknowledgment of their help in collecting the samples, and in carrying through most of the analyses is made to the members of the Extension staff in Plant Pathology at the Pennsylvania State College, and to Mr. James M. Stewart, respectively.

TABLE 1.—*Analysis and source of lime-sulphur samples arranged in order of specific gravity*

Sample number	Specific gravity	Polysulphide sulphur	Thiosulphate sulphur	Source
24	1.293	25.94	1.47	Home-boiled
9	1.283	23.68	2.56	Brand D
23	1.279	24.79	1.55	Brand E
4	1.279	22.73	4.19	Brand B
12	1.278	24.17	1.93	Brand B
28	1.278	23.13	2.09	Brand E
16	1.278	22.92	1.71	Brand E
29	1.272	22.91	1.71	Brand G
27	1.272	22.82	1.78	Brand F
26	1.270	22.95	1.86	Brand E
21	1.270	22.80	2.17	Unknown[a]
7	1.268	21.86	2.17	Brand C
17	1.268	21.78	1.78	Brand F
22	1.267	22.47	2.25	Brand C
18	1.266	21.93	2.02	Brand C
3	1.266	21.57	3.57	Brand A
8	1.265	21.43	2.40	Unknown[a]
14	1.263	22.75	2.17	Brand D
30	1.262	24.47	2.17	Brand E
6	1.262	22.58	2.71	Brand C
13	1.260	23.18	2.33	Brand D
10	1.259	21.48	2.17	Brand D
5	1.258	21.58	4.11	Brand C
20	1.258	21.28	2.09	Brand D
25	1.252	21.81	2.09	Brand D
11	1.250	22.20	2.48	Brand D
19	1.240	17.74	2.95	Home-boiled
1	1.235	15.65	6.05	Home-boiled
15	1.210	13.30	4.50	Home-boiled
2	1.182	10.90	5.27	Home-boiled

[a] Commercial but manufacturer not known.

diluting the concentrate so as to produce a given specific gravity after dilution be used there would be little difference between the two final sprays with regard to their polysulphide sulphur content; it is only when the operator follows the procedure of diluting his lime sulphur year after year to 1–75 or 1–100 or some other dilution, regardless of the specific gravity, that he may find his final spray less effective or more injurious than he hoped.

Thiosulphate Sulphur.—Table 1 indicates that there is a great difference in the thiosulphate-sulphur content of the various samples analyzed. The range of this constituent is between 1.71 per cent and 6.05 per cent, the samples with the lowest specific gravity and polysulphide sulphur content showing in general the highest concentration of thiosulphate sulphur. Statistically, however, there was no correlation between the thiosulphate contents and either the specific gravity or the polysulphide concentrations. Samples 3, 4, 5, and 19 are relatively high in thiosulphate sulphur.

The importance of the thiosulphate-sulphur concentration in lime-sulphur sprays is not too well understood, but there seems to be a general agreement that it is of little or no value as a fungicide, nor does there appear to be any evidence for considering it injurious to foliage.

Commercial vs. Homemade Lime Sulphur.—Five of the 30 samples of lime sulphur collected for this study were homemade; that is, they were boiled by the orchard operator. It is interesting and probably significant that the 4 samples lowest in polysulphides were homemade, and these same 4 samples were among the highest in thiosulphate sulphur. The average polysulphide-sulphur content of the 5 homemade samples was 16.70 per cent, while for the 25 commercial samples it was 22.61 per cent. Thiosulphate sulphur of the homemade samples averaged 4.05 per cent, while the average for the commercial samples was 2.32 per cent.

With one exception, it may be said that the homemade samples were decidedly inferior to the commercial samples. Peculiarly enough, the fifth sample of homemade lime sulphur (sample 24) was the best of the 30 samples examined. This indicates that, with proper care and equipment (in this case a steam heated kettle), lime sulphur of a quality equal or superior to commercial brands may be produced on the farm.

THE PENNSYLVANIA STATE COLLEGE,
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PHYTOPATHOLOGICAL NOTES

A Fungus Disease of Arceuthobium.—In 1935 Gill[1] gave a brief account of an apparently parasitic imperfect fungus, associated with the premature death of shoots of several forms of *Arceuthobium campylopodum* Engelm. in Washington and Arizona. In connection with a mistletoe survey for the Civilian Conservation Corps, the writer recently has had an opportunity to collect and observe the same fungus in several localities in Arizona on both *A. campylopodum* and *A. douglasii* Engelm. Examination of dried mistletoe collections, in the herbarium of the Division of Forest Pathology at Albuquerque, New Mexico, indicates that the disease may be quite widespread. What appears to be the same fungus has been found in herbarium material of *A. campylopodum* from various localities in Arizona, California, Oregon, Utah, and Washington and on *A. douglasii* from New Mexico. It was found on the following forms[1] of *A. campylopodum:* forma *abietinum,* f. *blumeri,* f. *cyanocarpum,* f. *divaricatum,* f. *microcarpum,* f. *tsugensis,* and f. *typicum.*

The disease is characterized by the appearance of yellowish-white, blister-like spots on the stems, which, in the later stages, erupt irregularly through the cuticularized epidermal layer, disclosing conspicuous white spore masses (Fig. 1). The lesions are most common at or near the nodes, and the stem is often completely girdled.

Both staminate and pistillate plants are attacked but the latter appear to be much more susceptible. The fungus apparently exerts some measure of biological control on mistletoe in areas where it has become well established. This is particularly true in connection with *Arceuthobium campylopodum* f. *cyanocarpum* in some localities in Arizona, where so many shoots

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[1] Gill, L. S. *Arceuthobium* in the United States. Trans. Conn. Acad. Arts and Sciences **32**: 111–245. 1935.

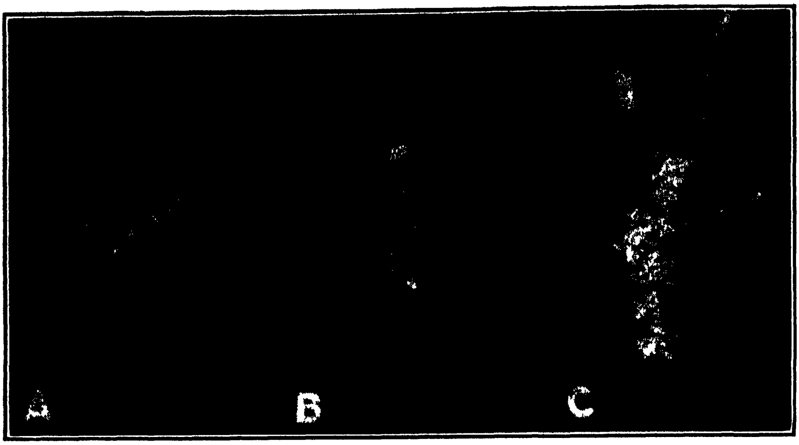

FIG. 1. Diseased shoots of *Arceuthobium campylopodum* f. *cyanocarpum*. A and B. Early stages with small light-colored lesions at nodes. C. Advanced stage with large, confluent eruption of white spore masses. Approximately × 2.

are prematurely killed that it is often difficult to find pistillate plants bearing fruits. Gill[2] noted a similar situation in connection with the disease on *A. campylopodum* f. *tsugensis* in the vicinity of Longmire, Washington.

The compact layer of hyaline, simple to ramose conidiophores arises from a stroma-like mass of hyphae in the cortex. The straight to slightly curved, 1- to 4-celled, cylindrical to fusiform conidia are borne singly on the tips of the conidiophores and measure 6–30 × 3–4 8 μ. The host tissues of both cortex and stele are filled with a profuse intercellular mycelium. Pending further studies the organism has been tentatively referred to the genus *Fusarium.*

Limited culture work indicates that the fungus may be quite specialized in its requirements. Mycelial growth is very slow and sparse on several agar media, but conidia are produced in abundance. Germination and subsequent growth are favored by relatively low temperatures. At room temperatures (21–24° C.) spores germinate very poorly and fail to continue growth after the germ tubes reach a length of about 100 μ, but in the refrigerator (5–7° C.) germination is good and colonies develop.

Further studies of the disease are under way.—DON E. ELLIS, Civilian Conservation Corps with the Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture, Albuquerque, New Mexico.

A Hybrid Cucumber Resistant to Bacterial Wilt.—Work on resistance to cucumber mosaic (cucumber virus 1) and bacterial wilt (*B. tracheiphilus* EFS.) was undertaken in 1929, by F. J. Pritchard and W. S. Porte, who crossed commercial varieties and Chinese Long, a mosaic-tolerant variety received from R. H. Porter of the Iowa Agricultural Experiment Station. In 1931 these hybrids, together with certain foreign and domestic varieties, were tested by Doolittle for resistance to mosaic and wilt. Tokio Long

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[2] See footnote 1.

Green proved equal to Chinese Long in mosaic tolerance and was somewhat resistant to bacterial wilt. Since it was definitely superior to Chinese Long in shape and color of fruit, a number of hybrids were made with standard varieties and the F_2 progenies tested at the U. S. Horticultural Station at Beltsville, Maryland, in 1934 and 1935. Several were markedly mosaic-tolerant under severe inoculation tests, and a few were wilt-resistant in the field. One, a hybrid of Tokio Long Green and Vickery Forcing, while not highly mosaic-tolerant, was sufficiently resistant to wilt to warrant further selection.

In 1936 no seed was obtained from late-selfed flowers of Tokio Long Green × Vickery Forcing plants that had survived to mid-August, because of damage from leaf-blight fungi. Seeds of open-pollinated fruits, however, were taken from plants surviving in September and the seed of each fruit carried as a separate line. In 1937, 5 replications on September 1 showed only 52 to 68 per cent wilt in certain selfed lines and in the open-pollinated field selections of 1936, as compared with 94 per cent in White Spine, a moderately wilt-susceptible variety, used as a control. Many self pollinations were made on plants of resistant lines in the field during August, 1937, but, again, the vines were so badly damaged by anthracnose and downy mildew that little seed was secured. All of this was planted in the greenhouse at once and the resulting progeny of each seed planted in the field in 1938 as a separate line in duplicate or quadruplicate plots. These trials proved that the

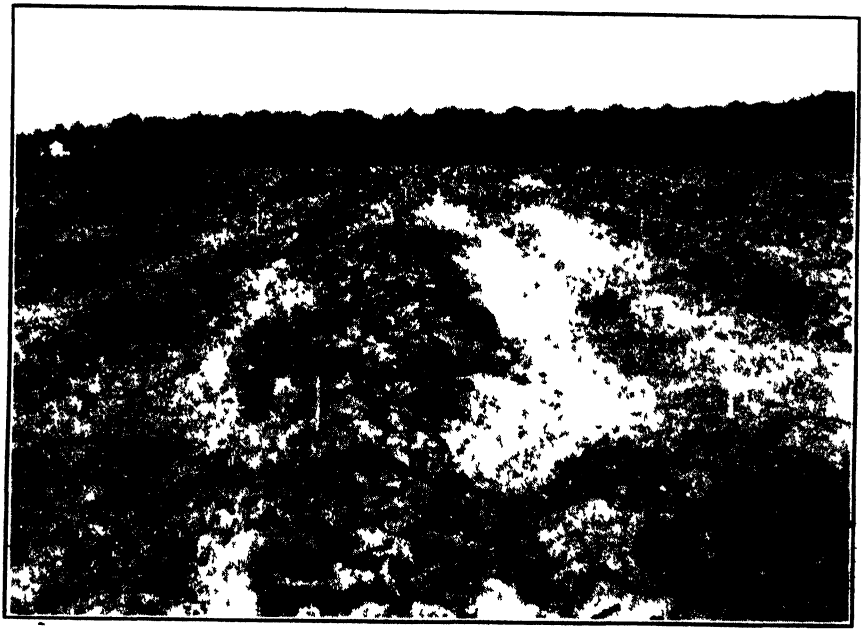

FIG. 1. Portion of field trials for resistance to bacterial wilt showing plots of the resistant Tokio Long Green × Vickery Forcing hybrid (lower foreground and center) with susceptible varieties on right and left. All missing plants had died of wilt. U. S. Horticultural Station, Beltsville, Md., August 19, 1938.

1937 field selections were of greatly increased resistance, since 14 lines, either entirely selfed or with one open-pollinated generation (1936), showed only 18 to 32 per cent wilt on September 13, variation within these limits not being statistically significant. On the same date White Spine showed 74 per cent wilt and highly susceptible foreign varieties 100 per cent. The actual crop loss was slight in the resistant lines, since only about 10 per cent of the plants were dead on August 22 (Fig. 1), while 30 per cent of the controls were dead on August 8, and 48 per cent on August 22.

In older resistant plants in the field the progress of the wilt organism appears to be so retarded that primary leaf infections often fail to become systemic and even an entire shoot, occasionally, may wilt without further infection of the plant. When resistant plants in the greenhouse are inoculated in the 2- or 3-leaf stage, they seldom survive indefinitely but the progress of the bacteria in the vessels appears to be definitely slower than in susceptible plants, and the degree of retardation is closely correlated with the relative resistance of the same lines in the field. Occasionally, such a plant has survived and produced fruit after being systemically infected, but in most instances plants that seem to recover will collapse suddenly and die from wilt after they reach the fruiting stage.

The Tokio Long Green × Vickery Forcing hybrid is not yet entirely satisfactory in horticultural type. The vines are vigorous, medium early, but only fairly prolific. Fruits are white-spined, smooth, cylindrical, rounded at the base and apex, and medium dark green with lighter tip stripes. When mature they are pale-cream color and about 8–10 × 2¾–3¼ inches. Certain other hybrids of more marked mosaic tolerance have shown some wilt resistance. Further work is in progress, particularly with reference to suitable pickling varieties. So far, however, all hybrids between pickling varieties and mosaic-tolerant sorts, such as Tokio Long Green and Chinese Long, have shown little evidence of wilt resistance.—S. P. DOOLITTLE, F. S. BEECHER AND W. S. PORTE, U. S. Horticultural Station, Beltsville, Maryland.

Notes on North American Pine-Oak Species of Cronartium on Castanea, Castanopsis, and Lithocarpus.—So far as known to the writer no pine-oak species of *Cronartium*, except *C. cerebrum* Hedgc. & Long, has been found in the United States in nature on any broadleaf host except *Quercus*. In July, 1925, the writer collected the telial stage of *C. cerebrum* on leaves of *Castanea dentata*[1] near Andrews and Asheville, North Carolina, and abortive telia on those of *C. pumila* near Asheville.

The first reported occurrence of *Cronartium quercuum* Miyabe in China was made by S. C. Teng,[2] who reported the occurrence of the uredial and telial stages on *Castanea mollissima* in the Nanking region in China. From Japan, the writer has received specimens of the uredia of *C. quercuum* on the leaves of *Castanea* as follows: collected by Ogawa on *C. crenata* at Komaba in the region near Tokyo, May 1929; on leaves of *C. mollissima*, collected by D. & H. near Takao, July 9, 1929.

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[1] Names of native species of trees are those of A. Rehder in his "Manual of cultivated trees and shrubs" 1937. The names of foreign species are those of A. Camus in "Les chataigniers. Monographie des genres *Castanea* and *Castanopsis*." (Paris) 1929.

[2] Teng, S. C. Fungi of Nanking II. Contr. Biol. Lab. Sci. Soc. China 8: 13. 1932.

At various times from 1909 to 1930 inoculations were made under controlled conditions in the pathological greenhouses at Washington, D. C., by the writer and his assistants, N. Rex Hunt and Glenn G. Hahn, with spore forms of several species of pine-oak *Cronartium*. Infection of the leaves of *Castanopsis chrysophylla* with *Cronartium cerebrum* was reported in 1911.[3] Infection of the leaves of *Castanea dentata, C. pumila*, and *C. sativa* with the urediospores of *Cronartium strobilinum* was reported in 1922.[4] Infection of the leaves of *Castanea dentata, C. pumila*, and *C. sativa* with urediospores of *Cronartium conigenum* was reported also in 1922.[5]

A summation is given in table 1 of the results of inoculations of species of *Castanea, Castanopsis*, and *Lithocarpus* with aeciospores and urediospores of *Cronartium cerebrum, C. conigenum, C. fusiforme* Hedgc. & Long, *C. strobilinum*, and the urediospores of *C.* sp., a species found on the leaves of evergreen oaks in California without a known aecial stage.

TABLE 1.—*Results of inoculations of species of Castanea, Castanopsis and Lithocarpus with five species of pine-oak Cronartium*[a]

Species inoculated	Species of *Cronartium*				
	C. cerebrum	*C. conigenum*	*C. fusiforme*	*C. strobilinum*	*C.* sp.
Castanea:					
alnifolia	–	–	–	–	–
alnifolia x *mollissima*	–	+	+	+	+
dentata	+	+	+	+	+
henryi x *mollissima*	+	0	+	0	0
mollissima	+	+	+	+	+
pumila	+	+	+	+	+
sativa	+	+	+	+	+
seguinii	–	0	–	0	0
Castanopsis:					
argentea	+	0	–	+	0
chrysophylla	+	0	+	+	+
delavaya	–	–	–	+	–
diversifolia	+	+	+	+	+
tribuloides	–	0	–	0	0
Lithocarpus densiflora	+	+	+	+	–

[a] –, no infection; +, infection; 0, no inoculation.

In all the infections reported in table 1, uredia, telia, or both resulted from the infection. In some species listed as uninfected necrotic lesions were formed on the leaves without spore production. Control plants did not become infected in any experiment.

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[3] Hedgcock, G. G. Notes on *Peridermium cerebrum* Peck and *Peridermium harknessii* Moore. Phytopath. 1: 131–132. 1911.

[4] Hedgcock, Geo. G., and Glenn G. Hahn. Two important pine cone rusts and their new cronartial stages, part 1, *Cronartium strobilinum* (Arth.) Hedgc. and Hahn. Phytopath. 12: 112. 1922.

[5] Hedgcock, Geo. G., and N. Rex Hunt. Two important pine cone rusts and their cronartial stages, part 2, *Cronartium conigenum* (Pat.) Hedgc. and Hunt. Phytopath. 12: 119. 1922.

In spite of the fact that Arthur[6] and others have not recognized the validity of the species given in table 1, it has been found that, in addition to differences shown when the species were originally described, they differ considerably in behavior on inoculated plants under similar greenhouse conditions and that the fruiting and spore forms of the different species vary in their measurements.—GEORGE G. HEDGCOCK, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

Isolation of Phytopathogenic Actinomycetes.[1]—Ordinary media are rather unsatisfactory for isolation of Actinomycetes from scab lesions on the tubers of potatoes and on the roots of other plants because of secondary organisms that commonly occur in such lesions. Taylor[2] suggested surface sterilization of the potato tubers with a calcium hypochlorite-sodium hydroxide solution and plating of the scab lesions on Waksman's egg-albumin agar. The disinfectant represses many bacteria and the medium inhibits "spreader" bacteria. However, his method of triturating the diseased tissues with a pestle and mortar invites fungus contamination.

The following technique has been found very satisfactory for isolation of Actinomycetes from potato-scab lesions and from Actinomyces lesions on the roots of other hosts in Michigan. A scabby tuber is thoroughly washed in tap water, immersed in 0.1 per cent $HgCl_2$ for 1 min. and rinsed under a tap. A scab lesion with the underlying tissue is cut out with a flamed scalpel and placed in a sterile, cotton-plugged, test tube. A heavy glass rod dipped in alcohol, flamed, and inserted into the tube is used to break up the diseased material. Thorough trituration is unnecessary. About 10 cc. of sterile water is then pipetted into the tube and the contents are shaken. One-tenth cc. of this suspension is further diluted with 10 cc. of sterile water. One-tenth to 1 cc. of this suspension in a Petri dish, plus about 20 cc. of medium, generally gives a satisfactory dilution. But, since the degree of the dilution will depend on the thoroughness with which the diseased tissue is macerated and the size of the scab lesion, it is advisable to pour plates of several dilutions.

In selecting a suitable medium for the separation of Actinomycetes from secondary organisms and contaminates, advantage was taken of the fact that Actinomycetes commonly grow well on media containing very small quantities of nutrients. The most satisfactory medium tried consists of 1 g. of glucose and 1 cc. of a 10 per cent solution of each of the following salts: KH_2PO_4, $NaNO_3$, KCl, and $MgSO_4 \cdot 7H_2O$; and 15 grams of agar per liter of distilled (or tap) water, pH adjusted to neutral. Most bacteria and fungi grow very poorly or not at all on this medium, while Actinomycetes grow well, the colonies usually becoming visible to the unaided eye in 2 or 3 days.

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[6] Arthur, J. C. Manual of rusts in the United States and Canada. 1934.

[1] Journal article No. 375 (n.s.) of the Agricultural Experiment Station of Michigan State College, published by permission of the Director.

[2] Taylor, C. F. A method for the isolation of Actinomycetes from scab lesions on potato tubers and beet roots. Phytopath. 26: 387–388. 1936.

The substitution of 1 g. per liter of soluble starch for glucose in the above described medium results in slightly better growth of Actinomycetes; but, with respect to inhibition of bacteria and fungi, the medium is somewhat inferior to that containing glucose. It seems possible, however, that some Actinomycetes that grow very poorly on glucose media may be phytopathogenic. In such cases the starch medium might be preferable. Media containing 0.1 per cent glucose or 0.1 per cent soluble starch, without the addition of the salt solutions or with only the nitrate, were somewhat inferior to the media containing small quantities of all 4 salts because of the repressing effect on the development of the Actinomycetes.—GLENN KENKNIGHT AND J. H. MUNCIE, Department of Botany and Plant Pathology, Michigan State College, East Lansing.

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Was Mayer the First to Record a Virus Disease of Plants?—There recently came to the writer's attention, from a most unusual source, a cut of what appears to be a virus disease of tulips. That, in itself, is not peculiar. The dates appearing on the cut, however, are of unusual interest.

Dr. Adolf Mayer (1886) gave us what appears to be the first description of a virus disease of plants when he described tobacco mosaic.[1] Iwanowski[2] first demonstrated in 1892 the filterability of the infective principle of the juices from mosaic-diseased tobacco plants.

The above mentioned cut appears on page 6 of *Portfolio No. 1* of The Old Print Shop, 150 Lexington Avenue, New York. Below the cut appears the caption, "Dr. Thornton's 'Temple of Flora' (1798–1808)." McWhorter[3] states that "broken" tulips are infected with two viruses. The tulips pictured in the cut show unmistakable evidence of "breaking."

Correspondence with Mr. Robert L. Harley, of The Old Print Shop, disclosed the following information concerning "Dr. Thornton's 'Temple of Flora' ":

> ". . . the engraving of tulips from 'Dr. Thornton's *Temple of Flora,*' which was illustrated in our recent pamphlet, was actually made between the dates 1798 and 1808 from a sketch or drawing of some sort made slightly earlier.
>
> "Dr. Thornton himself, we understand, made no drawings at all. He employed several different artists to work for him, and after the prints were made from these drawings, he assembled them and published the collection."

Can the original print be construed as sufficient evidence that such a virus disease existed in tulips at that early date? Is the evidence submitted sufficient to establish the fact that the first record of a virus disease of plants was known to have occurred between 1798 and 1808?—J. M. RAEDER, Assoc. Plant Pathologist, Idaho Agricultural Experiment Station, University of Idaho, Moscow, Idaho.

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[1] Mayer, A. E. Ueber die Mosaikkrankheit des Tabaks. Landw. Vers. Stat. 32: 451–467. 1886.

[2] Iwanowski, D. Ueber die Mosaikkrankheit der Tabakspflanze. Bull. Acad. Imp. Sci. de St. Petersbourg III, 35: 67–70. 1892.

[3] McWhorter, F. P. "Broken" tulips are diseased. Florists Exch. and Hort. Trade World 90: 15, 18. 1938.

BOOK REVIEWS

Pape, Heinrich. *Die Praxis der Bekampfung von Krankheiten und Schadlingen der Zierpflanzen.* 3. Aufl. 475 p., 8 col. pl., 336 fig. P. Parey (Berlin) 1939. Price Rm. 19 (subject to a 25% discount on foreign purchases).

It seems remarkable that, in the United States where the production of ornamental plants is a $100,000,000 plus industry, no complete manual of the diseases and pests affecting garden and greenhouse flowers, ornamental shrubs, and shade trees has yet appeared. Existing publications in this field are either books of limited scope or experiment-station bulletins devoted to only a few of the disease and pest problems that confront the gardener. In Germany a handbook covering this range of subjects has gone through two editions, and a 3rd revised and enlarged edition has recently appeared. It contains about 50 more pages and 33 more figures than the 2nd edition of 1936. As in the former editions, the plan is followed of presenting a general discussion of the extent of plant disease and pest losses, the agents of and factors in parasitism and disease, and the means and materials used in their control. Next, there is a "special section" devoted to (a) plant parasites and (b) animal pests, injurious in garden and greenhouse plant culture generally; and another "special section" (more than two thirds of the book) in which the diseases and pests of individual plant species are described. Hosts are listed alphabetically from *Abies* to *Zinnia,* though numerous genera of ornamental value are omitted; diseases (including virus, physiological, and of unknown cause) and pests (insects, nematodes, mollusks) are grouped according to the plant part attacked. The discussion of minor diseases and pests, and information of special mycological and entomological interest, are printed in reduced type to indicate their relative importance and to conserve space. Many of the illustrations are technically excellent and most are well reproduced. They are extremely helpful in recognizing the disease or injury with which one has to deal. There are indices listing (1) general and special literature in the field covered, (2) hosts by common and Latin names, (3) parasites similarly, and general subject matter.

The coverage of special literature is, in general, thorough and up-to-date. The reviewer would call attention to the omission of *Phytophthora megasperma* and *Plenodomus meliloti* on *Althaea,* concerning which competent scientific accounts have appeared.

American gardeners are probably not sufficiently "pest minded" as yet to make efficient use of a comprehensive book in this field, but to professional pathologists and entomologists who must deal with ornamentals this sort of book is a boon. Could our gardeners but avail themselves of the information thus presented in a foreign language, quite a number of Federal and State workers now serving as horticultural advisers to the general public could find a great deal more time for constructive work.—**Freeman Weiss.**

Gardner, V. R., F. C. Bradford, and H. D. Hooker, Jr. *Fundamentals of fruit production.* 2nd Ed., 788 pp., illus., $5.00. McGraw-Hill Book Company (New York).

Seventeen years ago the first edition of this work appeared. At that time it proved to be the most useful and up-to-date publication of its kind. The chapters on winter injury, frost protection, effects of surpluses and deficiencies of moisture, effects of mineral excesses and deficiencies, and other closely related subjects were very useful to plant pathologists. Plant pathologists were also supplied with a working knowledge of fruit production, which enabled them to understand more fully the parts played by soil moisture, nutrition, light duration, temperature, humidity, pruning, and a host of other factors on the incidence of disease.

The second edition consists of a great deal of the same material as presented in the first edition. Plant pathologists in general will find the sections "Water relations," "Nutrition," "Temperature relations of fruit plants," and "Geographic influences in fruit production" of special interest. The remainder of the book, devoted to horticultural practices and to fruit setting, is a valuable source of information to those specialists dealing with fruit pathology.

While this edition, like its predecessor, presents much interesting and valuable material from many sources, it still contains concepts and interpretations based on older work that have been changed by more recent researches.—**M. C. Goldsworthy,** Division of Fruit and Vegetable Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, Beltsville, Maryland.

REPORT OF SUMMER MEETING OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY, JUNE 20–23, 1939

The American Phytopathological Society held its summer meeting with the north Central Group of Phytopathologists at Milwaukee, June 20, and at Madison, Wisconsin, from June 21 to 23. President C. A. Dykstra and Dean E. B. Fred of the University of Wisconsin welcomed visiting scientists of all the botanical societies at a general session, June 21.

The scientific program was limited, in accord with numerous suggestions, to laboratory exhibits and field trips, except for joint sessions with other societies. These included sessions with the American Society of Plant Physiologists, on Tuesday and Wednesday afternoons, and a session of all botanical societies, Thursday morning, I. E. Melhus presiding. At this meeting the Society was represented by E. C. Stakman, University of Minnesota, who gave an invitation paper on "Variability among the smut fungi." The Forest Products Laboratory, the laboratories of plant pathology and of certain other plant-science departments of the University of Wisconsin were open for inspection and for demonstration, both of techniques employed in research and of results obtained. Facilities were offered for a number of field trips to experimental plots and to surrounding areas of scientific interest.

A program, with morning, afternoon, and evening schedules of "non-technical activities," was arranged for visitors who accompanied Society members. This included a tea Wednesday afternoon, given by the wives of botany and plant-pathology staff members.

The Phytopathology dinner, Wednesday evening, was shared by 115 members and friends. G. W. Keitt introduced C. R. Orton, President of the Society, who conducted a short business meeting of the North Central Group. H. W. Anderson, University of Illinois, invited the pathologists to hold their 1940 summer meeting in southwestern Illinois. The committee chosen to make necessary arrangements includes H. W. Anderson, University of Illinois, chairman, I. E. Melhus, Iowa State College, and C. R. Tucker, University of Missouri. The program was concluded by moving pictures in natural color of Wisconsin fungi and wild flowers, presented by the State Conservation Commission of Wisconsin.

ANNOUNCEMENT

OF A

BIOCLIMATICS-BIOMETEOROLOGY SUBSECTION OF BIOLOGICAL ABSTRACTS

To all engaged in research or instruction in plant pathology or other life sciences the announcement will be welcome that BIOLOGICAL ABSTRACTS is undertaking a more complete abstracting and segregation of current research literature on weather and climate in relation to agriculture and biology. A new subsection, *Bioclimatics, Biometeorology,* within the section *Ecology* is now appearing under the editorship of Robert G. Stone of the Blue Hill Observatory, Harvard University.

Though often devoting much attention to the same fundamental natural forces, many diverse research groups which have sprung up in various subject matter fields still work in practical isolation from one another, belonging to separate societies and publishing in different journals. BIOLOGICAL ABSTRACTS aims now to help these groups in applying common ideas and methods to the study of situations that are basically similar, as, for example, where techniques and concepts derived from a study of the influence of weather factors on the inception and epidemiology of animal or human diseases are likely to have a very high transfer value as applied to studies of the spread or survival of plant diseases. In situations like this an abstracting journal of broad scope, like BIOLOGICAL ABSTRACTS, appears admirably suited to the sort of synthesis of fundamental knowledge which is demanded. In inaugurating this section, gathering together items from wide sources dealing with climatic factors in relation to living things, BIOLOGICAL ABSTRACTS is carrying on only one of the functions for which its nonprofit service to scientists was originally created, viz., to provide biologists with easier access to the literature of borderline fields.

Under the current sectional publication plan, which is to be continued, this bioclimatic material will be found, at present, not only in Section A—*Abstracts of General Biology,* but also under Section B—*Abstracts of Experimental Animal Biology,* Section D—*Abstracts of Plant Sciences,* and Section E—*Abstracts of Animal Sciences.* Section D is the separately published subject group which includes abstracts of the literature on plant pathology as well as that on plant physiology, anatomy, and systematics, and agronomy, horticulture, forestry, ecology, and other related lines.

THREE SPECIES OF PYTHIUM WITH LARGE OOGONIAL PROTUBERANCES

CHARLES DRECHSLER

(Accepted for publication July 20, 1939)

Among 15 members of the genus *Pythium* that I (5) presented nearly a decade ago as species new to science, were included 2 forms, *P. mastophorum* and *P. polymastum,* that were set forth at the time as being apparently most directly related to *P. megalacanthum* de Bary. Owing to the circumstance that *P. megalacanthum* has since then been cited in connection with injury to various economic plants in different countries, an increased possibility seems in prospect that my 2 forms may become rather variously understood in accordance with diverse applications of de Bary's binomial. To abate in some degree whatever occasion for error may arise, it appears expedient to supplement my earlier diagnoses with illustrated discussion of the more important of the relevant morphological and developmental details,—the illustrations, to facilitate comparison, having been prepared for reproduction at magnifications uniform with similar figures given earlier in papers on congeneric parasites (6, 7, 9). Such discussion likewise is devoted to a third fungus, which has been dealt with as *P. megalacanthum* more extensively in recent literature than any other, and which, besides, in its structural features, presents so obvious a parallelism with both *P. mastophorum* and *P. polymastum* that its intimate relationship to these forms seems unquestionable.

PYTHIUM MASTOPHORUM

Though numerous cultures derived from a wide variety of affected vegetable structures have been examined during the 12 years since *Pythium mastophorum* was isolated from a discolored root of an unthrifty specimen of English daisy, *Bellis perennis* L., collected in the District of Columbia, the fungus has not yet been obtained from diseased material a second time. The species could not well have been overlooked in examinations of planted plate cultures, as, even previous to the development of its reproductive bodies, the characteristically haphazard disposition of its mycelial elements contrasts rather markedly with the more orderly arrangements of hyphae usual for most root-inhabiting oomycetes. Opportunity has been lacking for determining to what extent the fungus may be associated with rootlet decay of the English daisy, as diseased specimens of that ornamental have not again become available for use in the preparation of cultures.

Asexual reproduction of the fungus may be induced by shallow irrigation of well-nourished, actively growing mycelium. Such irrigation is readily accomplished by removing small slabs from a Lima-bean-agar plate culture to a sterile Petri dish and adding sterile distilled water until the liquid moistens, yet does not seriously flood, the upper surface of the slabs. As nutrient substances tend to diffuse out from the rich substratum, thereby encouraging a continuation of vegetative growth, it has often proved advantageous to replace the water at intervals. The manipulation required for these changes usually results in the introduction of putrefactive bacteria, which, in the course of 1 or 2 days, multiply so as to attain a concentration decidedly unfavorable for zoospore production by most root-rotting oomycetes. *Pythium mastophorum,* however, would seem little repressed in its asexual reproductive development as long as bacterial contamination remains fairly moderate, for usually, it continues to give rise to zoosporangia during the third and fourth days after the irrigation treatment is begun.

The zoosporangia, usually borne terminally (Fig. 1, A, B), or subterminally (Fig. 1, C, D, E), though sometimes occupying definitely intercalary positions (Fig. 1, Q, R), are of the subspherical type and have approximately the same dimensions as the homologous bodies, for example, of *Pythium irregulare* Buism. and *P. mammillatum* Meurs. Nevertheless, they would not readily be mistaken for reproductive bodies of the two more familiar parasites, owing partly to a perceptible tendency for them to assume an oblate ellipsoidal (Fig. 1, A–E) rather than a prolate ellipsoidal shape, and partly to a darker, less translucent consistency of their protoplasmic contents. In the latter respect they suggest somewhat the appearance of young oogonia or globose gemmae belonging to certain relatively delicate members of the Saprolegniaceae.

After an individual sporangium has been delimited from its supporting hypha by the deposition of 1 or 2 septa, it puts forth an evacuation tube, sometimes from an equatorial position (Fig. 1, F, G), sometimes from an apical position (Fig. 1, H, I), and sometimes from a basal position (Fig. 1, P). Sooner or later a large vacuole appears within the sporangium, and a refractive gelatinous cap is formed at the tip of the evacuation tube (Fig. 1, I). These changes presage discharge of the protoplasmic material into a vesicle resulting from inflation of the apical cap. In the vesicle cleavage of the granular material wholly after the fashion usual in the genus *Pythium* brings about its conversion into relatively large biciliate zoospores (Fig. 1, J, JJ, K–N). On being liberated through rupture of the vesicle, the zoospores swim about for a variable period in a slow, seemingly deliberate manner, and eventually round up into subspherical cysts having an average diameter of approximately 13 μ.

Proliferous development of sporangia has never been observed in any material of *Pythium mastophorum.* Very frequently, indeed, the portions of sporangiferous filaments adjacent to empty sporangia are likewise devoid of contents (Fig. 1, O–X). Irregularities in development of asexual reproductive apparatus have been noted in this species as in many other oomycetes: illustrative instances being represented often in excessive contortion of the evacuation tube (Fig. 1, G), in a somewhat branched condition of that tube (Fig. 1, J, M), and in production of a second tube following frustration of the first (Fig. 1, L).

Sexual organs are formed abundantly and promptly when *Pythium mastophorum* is grown on maize-meal agar. The young oogonia make their ap-

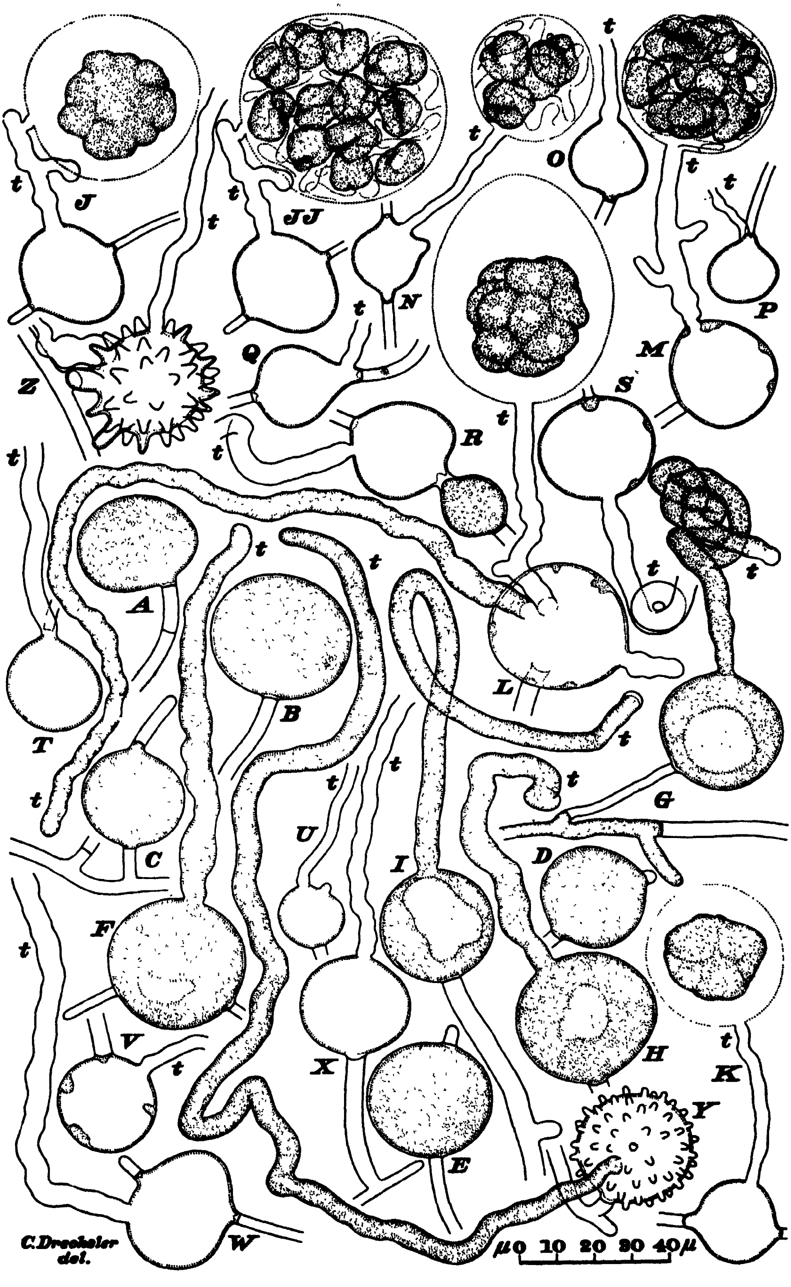

FIG. 1. Asexual reproductive apparatus of *Pythium mastophorum* formed on irrigated Lima-bean agar, and, with the aid of a camera lucida, drawn to a uniform magnification; ×500 throughout. A–E. Full-grown sporangia. F–I. Sporangia, each of which has put forth an evacuation tube. J, JJ. Sporangium showing respectively an early and a late stage in the fashioning of zoospores within the vesicle. K–N. Sporangia showing successively later stages in zoospore development. O–X. Empty sporangia, after escape of zoospores. Y. Oospore germinating by production of a long evacuation tube. Z. Empty oogonium, after germination of oospore by production of zoospores. (t, evacuation tube in F–Z.)

pearance in the substratum as globose bodies, usually borne terminally on short lateral branches that are often somewhat bent or contorted. Like the sporangia, they contain very densely granular protoplasm, the meager translucence of which gives them a darkish aspect. At a relatively early stage in the enlargement of the individual female organ, the usually single antheridium begins to develop in contact with it as a saccate or lobate expansion terminating a filament not closely connected with the oogonial hypha. The 2 sexual structures and the filaments supporting them are usually engaged with one another to a greater or lesser extent, the engagement being promoted at times by the presence of short diverticulations (Fig. 2, A, B, C). Before the oogonium has quite attained definitive size it puts forth protuberances, now in scattered arrangement, now in closer, bristling array. At first these protuberances may be hemispherical (Fig. 2, D, E), but soon they mostly become drawn out distally into broadly conical (Fig. 2, F) or even somewhat mammiform shapes (Fig. 2, G). After withdrawal of protoplasmic contents from them, the protuberances are revealed as characteristically thick-walled modifications with lumina often very markedly narrowed at the apex (Fig. 3, A–D). Following delimitation of both oogonium and antheridium through insertion of basal septa, the latter organ intrudes into the former a rather broad fertilization tube by means of which it delivers up its contents. A familiar sequence of developmental changes brings into existence an oospore that, at maturity, is provided with a wall of moderate thickness, and shows the internal structure most usual among species of *Pythium*,—its single large central reserve globule being surrounded by a granular parietal layer wherein a single refringent body, mostly oblate ellipsoidal in shape, is discernible (Fig. 3, A–D).

Degeneration of sexual apparatus is sometimes virtually absent in maize-meal agar cultures of *Pythium mastophorum*, while at other times, in the same medium, fully half of the oogonia fail to yield good oospores. Frequently only a portion of the granular material within an oogonium is lost, the remainder being utilized in endogenous formation of a smaller secondary oogonium, which is usually furnished with correspondingly fewer and smaller protuberances, and gives rise to a proportionately smaller oospore. Now and then similar partial degeneration in a secondary oogonium is concomitant with endogenous development of a tertiary oogonium, within which an oospore of small dimensions, yet of correct internal structure, may be borne. Except for a brief passage devoted wholly to a description of the secondary and tertiary oogonia, the diagnosis of *P. mastophorum*, for better comparison with related species, avoids consideration of these bodies and of the oospores formed in them, its statements on ordinary dimensions of oogonium, antheridium, and oospore being based on measurements of completely normal structures found in maize-meal-agar cultures that showed practically no degeneration of sexual apparatus. Normal and abundant sexual development of the fungus is accompanied generally by a dimensional variability that, in comparison with variability in some congeneric forms, may be re-

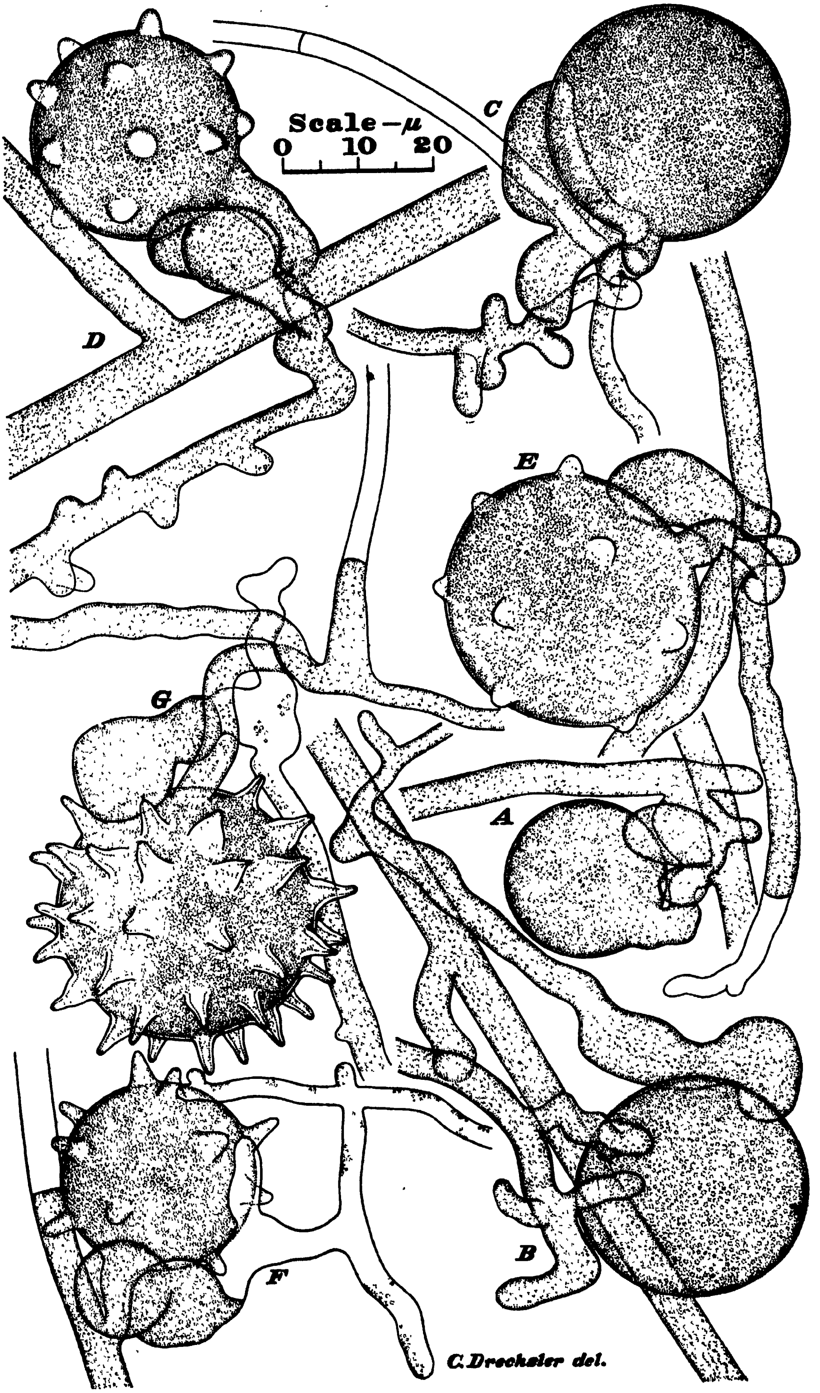

FIG. 2. A–G. Immature units of sexual apparatus of *Pythium mastophorum* formed in maize-meal-agar plate cultures, and, with aid of a camera lucida, drawn to a uniform magnification; × 1000 throughout.

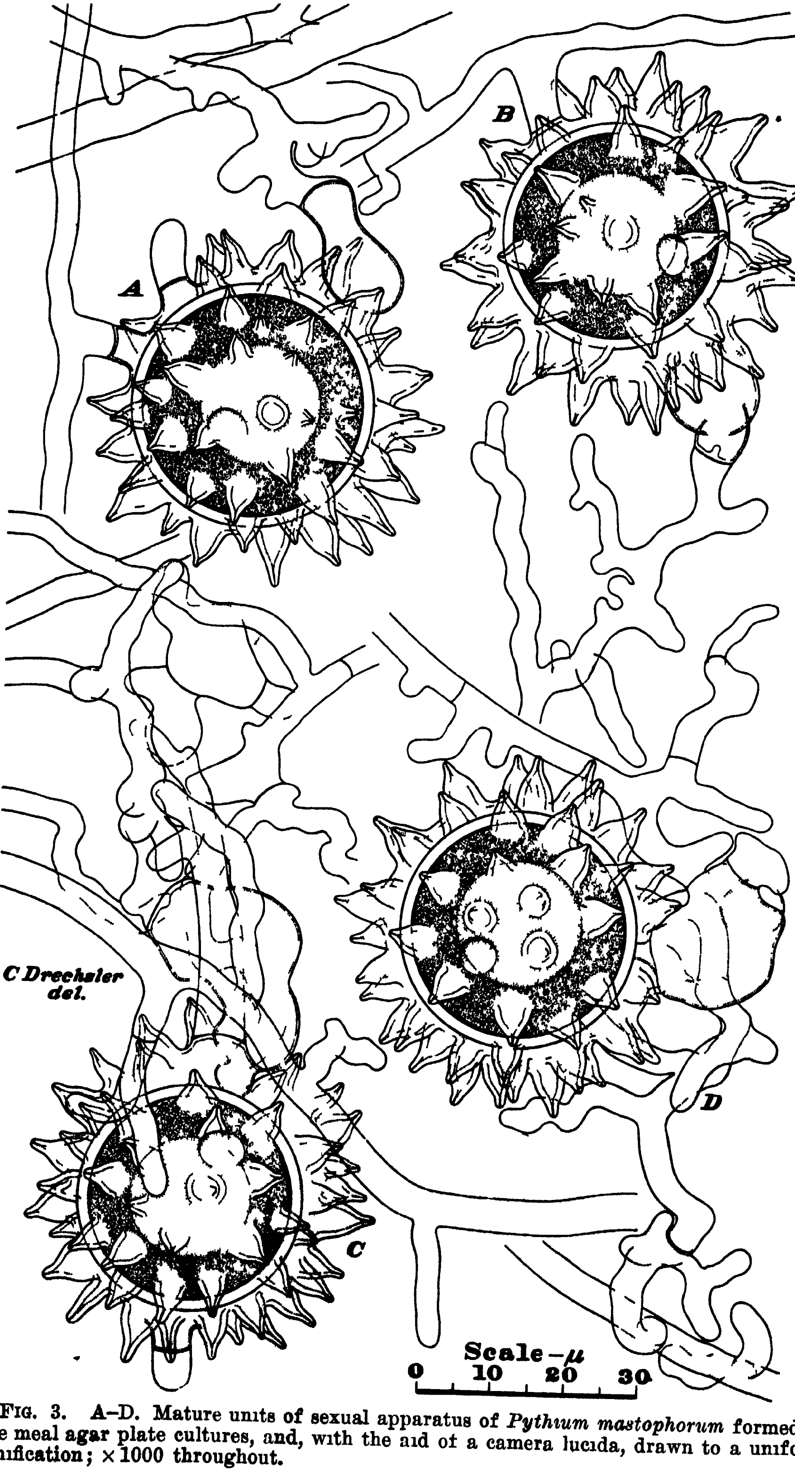

FIG. 3. A–D. Mature units of sexual apparatus of *Pythium mastophorum* formed in maize meal agar plate cultures, and, with the aid of a camera lucida, drawn to a uniform magnification; ×1000 throughout.

garded as moderate. Thus the 200 values for diameter of oogonium (exclusive of protuberances) from which was computed the average for this dimension given in the diagnosis—values obtained by measuring specimens selected at random—showed, when expressed to the nearest micron, a distribution as follows: 25 μ, 1; 30 μ, 3; 31 μ, 6; 32 μ, 15; 33 μ, 26; 34 μ, 27; 35 μ, 43; 36 μ, 24; 37 μ, 24; 38 μ, 15; 39 μ, 9; 40 μ, 6; 41 μ, 1. And the 200 values for oospore diameter from which was computed the average for that dimension—values obtained by measuring the oospores contained within the 200 oogonia—showed, when expressed to the nearest micron, the following distribution: 21 μ, 1; 22 μ, 1; 24 μ, 4; 25 μ, 5; 26 μ, 6; 27 μ, 23; 28 μ, 29; 29 μ, 46; 30 μ, 48; 31 μ, 28; 32 μ, 5; 33 μ, 3; 35 μ, 1.

Since *Pythium mastophorum* is usually rather reluctant to form zoospores, it is somewhat surprising that, when asexual reproduction occurs in irrigated preparations, a considerable proportion of such oospores as happen to have been produced simultaneously with the asexual sporangia likewise germinate by production of zoospores. The events associated with this type of germination follow the sequence earlier set forth in my account of *P. acanthicum* Drechsl. (9, p. 402).

PYTHIUM POLYMASTUM

Pythium polymastum came into my hands in a pure culture received with 13 other fungous cultures in May, 1925, from the late Doctor G. P. Clinton, in whose laboratory at the Connecticut Agricultural Experiment Station, New Haven, Conn., it was stated to have been isolated on April 1, 1921, from lettuce, *Latuca sativa* L. Although decaying roots of lettuce obtained from sickly plants in greenhouses both at the Arlington Experiment Farm, Arlington, Va., and at the U. S. Horticultural Station, Beltsville, Md., have on several occasions during more recent years been examined microscopically, the characteristic sexual bodies of the fungus could not be recognized in any affected tissues. The fungus has never been obtained when diseased lettuce roots of such origin were planted on agar media in Petri dishes; nor, indeed, has it come to light following similar treatment of discolored roots or rootlets from numerous other phanerogamic plants undertaken by me during a period of 18 years. To my knowledge the species has not been isolated from any natural source a second time. For some years, however, Clinton's original strain, as also the one and only strain of *P. mastophorum* hitherto brought into pure culture, has been widely available to mycologists at the Centraalbureau voor Schimmelcultures in Baarn, The Netherlands.

Growing in pure culture on maize-meal agar, *Pythium polymastum* shows approximately the same moderate rate of mycelial extension as *P. mastophorum;* and reveals, too, the same characteristically random disposition of its vegetative filaments. Although its vegetative hyphae exceed in width those of the form isolated from roots of the English daisy, the difference with respect to coarseness is not a conspicuous one. Because of their meager display of delicate branches both species must be reckoned among the coarser members of the genus *Pythium.*

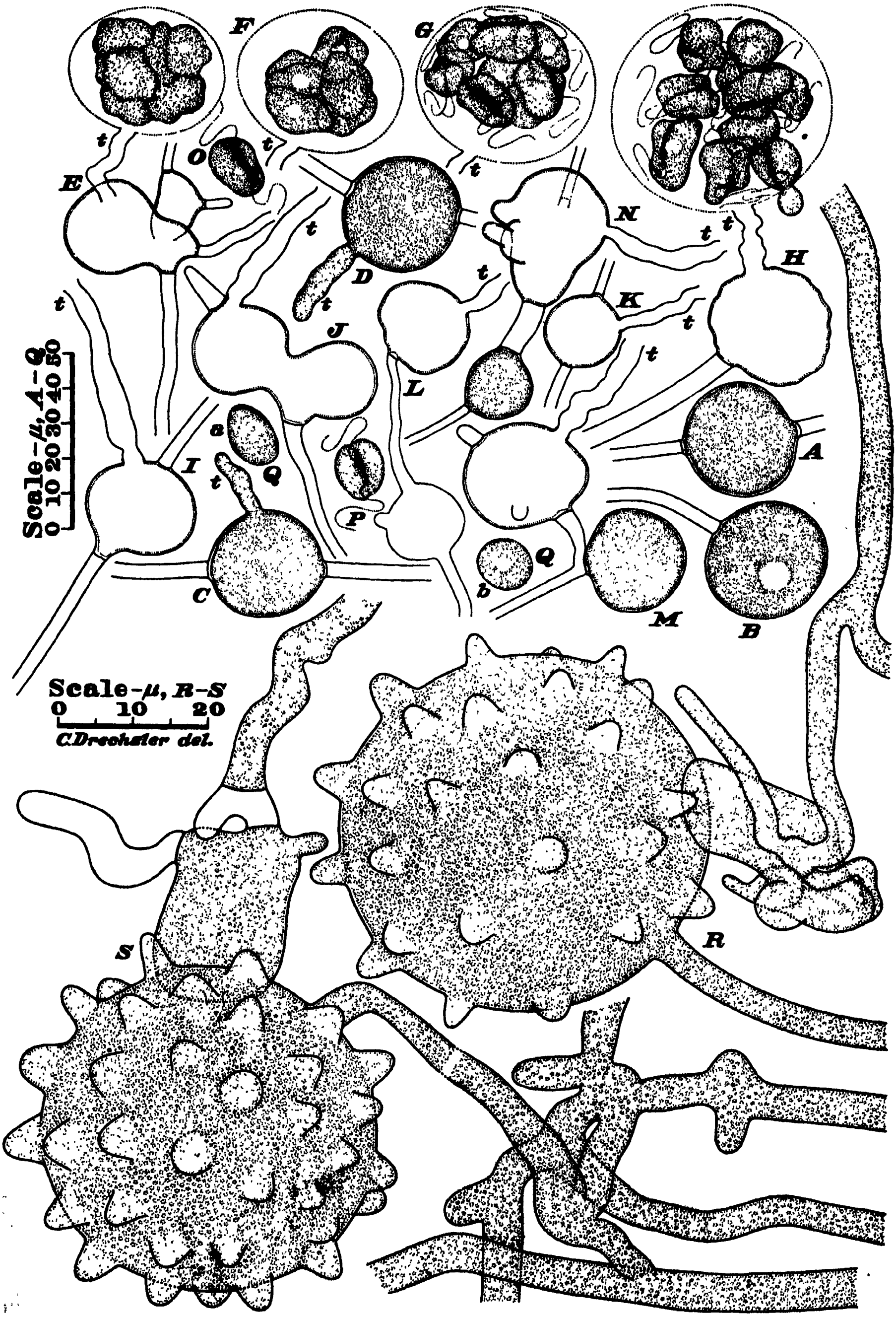

FIG. 4. *Pythium polymastum,* drawn with aid of a camera lucida. A–Q. Asexual reproductive apparatus formed on irrigated Lima-bean agar; ×500: A, B, Full-grown sporangia; C, D, Sporangia, each with an elongating evacuation tube; E, F, G, Sporangium, showing 3 successive stages in the fashioning of its zoospores; H, Sporangium, showing a zoospore in act of escaping; I–N, Empty sporangia; O, P, Motile zoospores; Q, a, b, Zoospores rounding up; t, evacuation tube in C–N. R, S. Immature units of sexual apparatus formed in maize-meal agar; ×1000.

Zoospore production is not readily induced in *Pythium polymastum,* but has nevertheless been obtained several times by carefully irrigating slabs excised from Lima-bean-agar plate cultures well permeated with young mycelium. The sporangia, like those of *P. mastophorum,* appear rather tardily as somewhat opaquely darkish bodies occurring in either intercalary (Fig. 4, A) or terminal (Fig. 4, B) positions. For the most part they are of subspherical or oblate ellipsoidal shape, yet irregular specimens are so frequent that they can hardly be considered exceptional. In some instances, for example, the sporangium is composed of 2 connected lobes (Fig. 4, E, J), or, again, it consists of a main swollen structure together with a number of dome-shape diverticula (Fig. 4, N). The evacuation tube (Fig. 4, C–N: t) may arise from an equatorial position (Fig. 4, C, D) or from a position closer to the basal or distal end (Fig. 4, I, J). On attaining a length sometimes less (Fig. 4, H, L) but at other times greater (Fig. 4, I) than the diameter of the sporangium, it yields at the tip to permit escape of the sporangial contents into a vesicle, where they undergo transformation into biciliate zoospores wholly through the development generally characteristic of species of *Pythium* (Fig. 4, E, F, G). Escape of the swimming spores ordinarily ensues from extensive disintegration of the vesicle as is usual in congeneric forms; though a few instances came under observation where a relatively small opening served as exit for one or several of the motile spores (Fig. 4, H) before the vesicular membrane—evidently one of more than customary toughness—gave way extensively. The empty sporangial envelope not infrequently shows a relaxed undulant contour (Fig. 4, H, L), but, even where irregularity of outline is not clearly noticeable (Fig. 4, E, I, J, K, M, N), some reduction in volume may be presumed to have accompanied loss of contents.

The zoospores of *Pythium polymastum,* after swimming about in a stately manner (Fig. 4, O, P), come to rest (Fig. 4, Q, a) and round up into cysts (Fig. 4, Q, b) with an average diameter of 15 to 16 μ. They are, indeed, the largest zoospores I have ever seen in any species of *Pythium.* Despite their extraordinary dimensions they seem clearly exceeded in size by the zoospores of *P. megalacanthum,* which, according to de Bary's account (2), round up into cysts with an astonishing average diameter of 18 to 20 μ. Since zoospores generally appear little given to pronounced dimensional variability, it is believed that a difference of 3 or 4 μ in average width of these bodies would in itself set apart Clinton's fungus as a species different from that of de Bary, notwithstanding certain obvious resemblances, which had led me at first to hold the two identical,—an error whereby the fungus under consideration came to be cited as *P. megalacanthum* in a paper by Harter and Whitney (10). Additional ground for objecting to the earlier tentative identification derives from the absence of any proliferous tendency in the zoosporangial development of *P. polymastum* under cultural conditions that evoked unmistakable proliferation in *P. anandrum* Drechsl.,—assuredly a fungus not disposed to produce zoosporangia at all abundantly.

When cultivated on maize-meal agar, *Pythium polymastum* usually begins its sexual reproduction 3 or 4 days after active mycelial growth has been

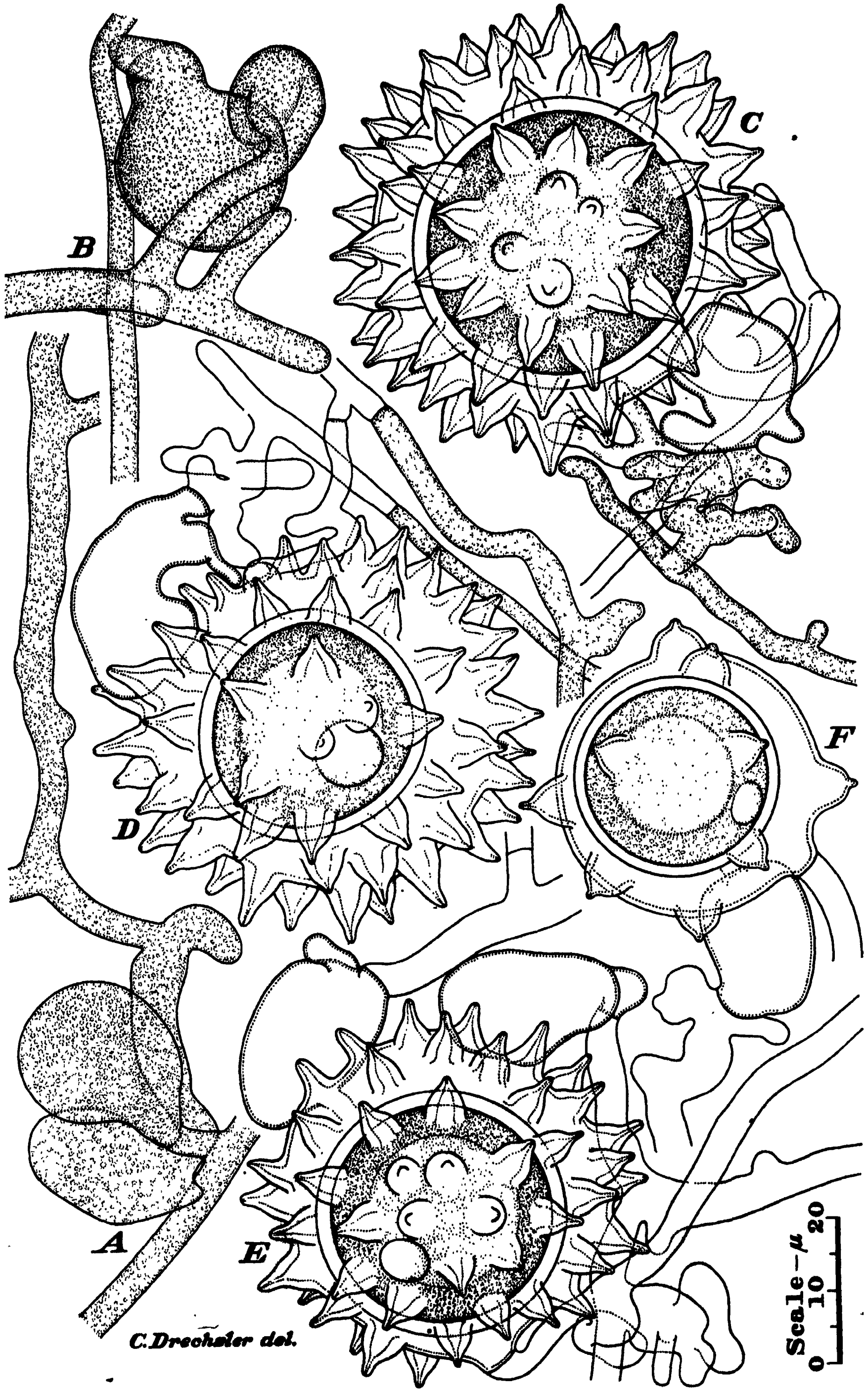

FIG. 5. Sexual apparatus of *Pythium polymastum* formed in maize-meal-agar plate cultures, and, with aid of a camera lucida, drawn to a uniform magnification; ×1000 throughout. A, B. Units at a very early stage of development. C–F. Mature units.

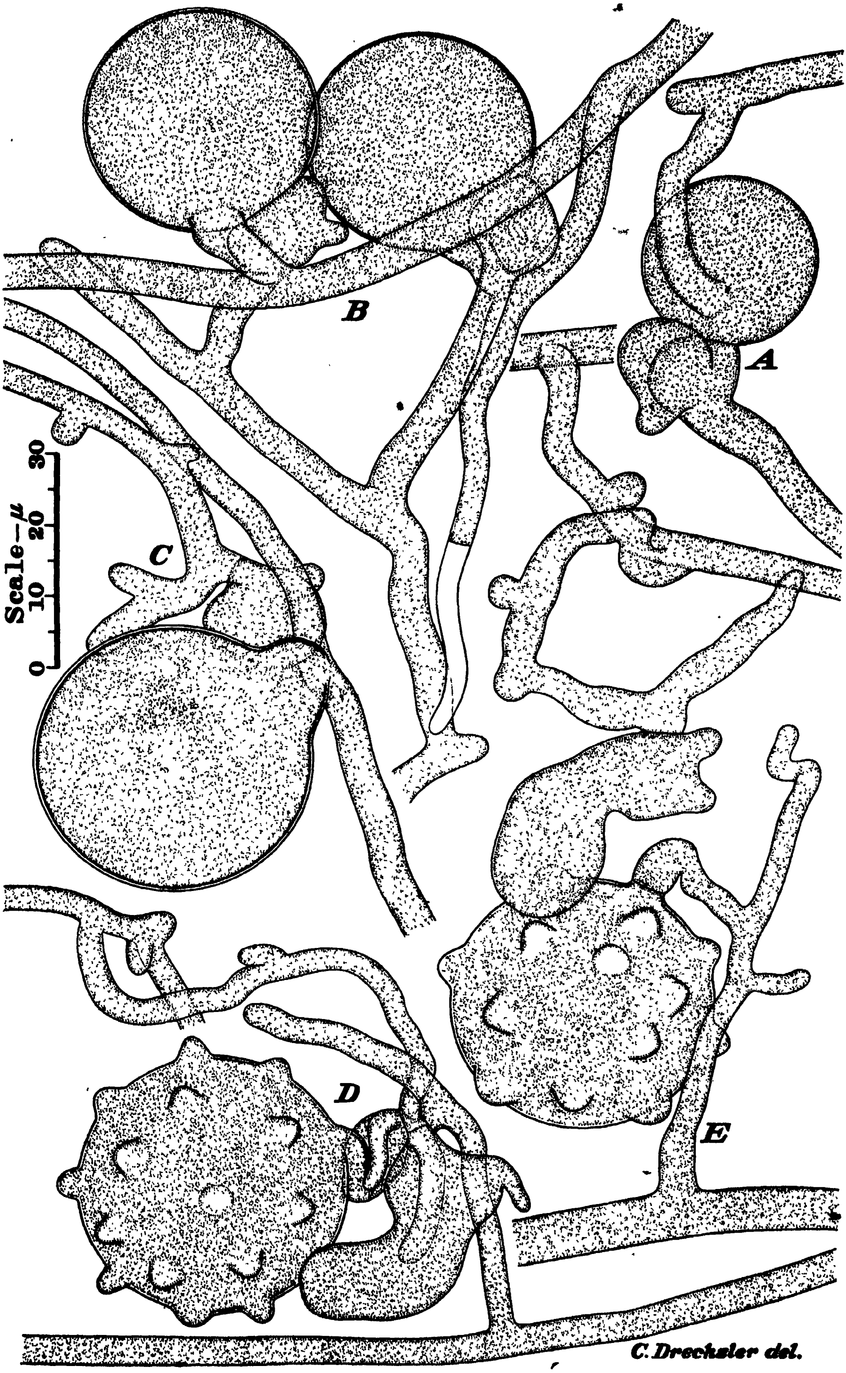

FIG. 6. A–E. Immature sexual apparatus in various stages of development, found in maize-meal-agar cultures, and, with the aid of a camera lucida, drawn to a uniform magnification; × 1000 throughout.

initiated. The oogonia first become visible as somewhat opaquely darkish globose bodies that are either borne terminally on relatively short branches (Fig. 5, A, B; 6, A, B) or are sessile and lateral on longer mycelial filaments (Fig. 6, C). From a very early stage the female structure is found in contact with a young antheridium, which consists often of a bulbous enlargement borne terminally or laterally on a hyphal element not closely connected with the oogonial filament (Fig. 5, A). Occasionally, 2 antheridia are present (Fig. 5, E). The sex organs remain continuous with the mycelium as they increase in size through migration of protoplasmic material into them. During the final stages of expansion the oogonium puts forth a variable number of rather wide proturbances (Fig. 4, R, S; 6, D), and then is delimited by the deposition of a basal septum (Fig. 6, E). At about the same time (Fig. 6, E), or frequently even a little earlier (Fig. 4, R, S), the antheridium, whose growth has in many instances been accompanied by a greater or lesser development of lobes, protuberances, or diverticulations, also is delimited by the laying down of a basal cross-wall. Soon the processes through which the single oospore is brought into being are inaugurated by noticeable contraction of the female protoplast away from its sculptured enveloping wall, intrusion of a stout fertilization tube into the oogonial chamber, and transfer of the antheridial contents.

The withdrawal of protoplasmic material from the oogonial envelope brings into clearer view marked variations among the protuberances with respect to shape, thickness of wall, and width of lumen. In some instances these excrescences are represented by narrow, cylindrical, thin-walled digitations 10 to 30 μ in length, which, for the most part, are bluntly rounded at the tip, yet which, again, may become expanded terminally and bear smaller secondary protuberances along the expanded portion; in other equally numerous instances, they are represented by small cusps with greatly reduced lumina. As, however, the more bizarre variations in design of the protuberances seem to be associated almost always with serious oogonial deterioration, their significance must be discounted generously in any sound characterization of the species. In cultures wherein degeneration is reduced in such measure that most units of sexual apparatus yield good oospores the oogonial protuberances appear more usually to be of conical or mastoid shape; the lumen, in most instances, being fairly wide in the broad, thick-walled basal part, but becoming conspicuously narrowed in the apical prolongation (Fig. 5, C–F). The number of protuberances present on oogonia with good oospores varies greatly: less than a dozen being visible in the upper and equatorial aspects of some comparatively smooth envelopes (Fig. 5, F), though, usually, scores are crowded together in the corresponding portions of the more elaborately sculptured envelopes (Fig. 5, C–E).

Despite their relatively large dimensions, the oospores of *Pythium polymastum* show the internal organization familiar among species of *Pythium* other than *P. helicoides* Drechsl. and its allies. At early maturity the parietal protoplasmic layer surrounding the homogeneous central reserve globule reveals a rather finely granular texture (Fig. 5, C–F). Examination after 6 or 8 weeks of storage at a temperature of approximately 5° C. shows usually a perceptible increase in size of the reserve globule, with an equal decrease in thickness of the parietal layer. The parietal layer, moreover, now shows coarser texture, its constituent granules having attained a diameter of about 0.5 μ; so that, except mainly for a less regular arrangement of the granules, the resulting appearance is reminiscent of the oospores of many saprolegniaceous forms, including all terricolous species of *Aphanomyces*. As in various congeneric forms, many oospores, after a prolonged period of dormancy, show imbedded in the parietal layer from 2 to 4 refringent bodies of somewhat smaller dimensions than the single large subspherical or oblate ellipsoidal refringent body found at early maturity (Fig. 5, C–F).

The fungus appears somewhat exacting in its nutritional requirements for proper sexual reproduction. Some culture media, including maize-meal-decoction agar, that allow abundant development of good oospores by the several *Pythium* species commonly associated with damping-off, will permit development of good oospores by *P. polymastum* in perhaps only about 5 or 10 oogonia out of every hundred such structures produced. Addition to an agar medium of a considerable amount of very finely ground maize meal usually brings about marked reduction in oogonial degeneration, with the result that as many as half of the units of sexual apparatus produced may then form oospores with correctly organized contents. Partial degeneration of oogonia, with endogenous development of smaller secondary oogonia that function successfully in forming good, though usually rather small, oospores, occurs in cultures of *P. polymastum* much as in cultures of *P. mastophorum*.

The information on oogonial dimensions submitted in the diagnosis of the species was based on measurements of 200 oogonia produced in a maize-meal-agar culture that had more than ordinary merit with respect to normality of sexual reproduction. The specimens measured were free even of partial degeneration, all having produced correctly organized oospores without intervention of any secondary oogonium. Measurements of the 200 oospores in question provided the metric data underlying the statements pertaining to dimensions of the oospore. In order to remedy in some part the irregular distribution of values that was evident when both sets of measurements were divided into classes having a range of only 1 μ, an equal number of additional measurements have been made with similar precautions against unnecessary incorporation of effects due to degeneration. The combined 400 measurements show a distribution of values for diameter of oogonium (exclusive of protuberances), expressed to the nearest micron, as follows: 26 μ, 2; 27 μ, 1; 28 μ, 4; 29 μ, 1; 30 μ, 1; 31 μ, 3; 32 μ, 4; 33 μ, 8; 34 μ, 4; 35 μ, 8; 36 μ, 12; 37 μ, 10; 38 μ, 10; 39 μ, 18; 40 μ, 16; 41 μ, 16; 42 μ, 22; 43 μ, 13; 44 μ, 37; 45 μ, 27; 46 μ, 29; 47 μ, 27; 48 μ, 29; 49 μ, 33; 50 μ, 20; 51 μ, 12; 52 μ, 13; 53 μ, 8; 54 μ, 4; 55 μ, 5; 56 μ, 1; 58 μ, 1; 59 μ, 1. The 400 oospores contained within the oogonia measured showed a distribution of values for diameter, expressed to the nearest micron, as follows: 21 μ, 1; 22 μ, 4; 23 μ, 2; 24 μ, 3; 25 μ, 8; 26 μ, 7; 27 μ, 9; 28 μ, 10; 29 μ, 6; 30 μ, 15; 31 μ, 15; 32 μ, 26; 33 μ, 20; 34 μ, 25; 35 μ, 34; 36 μ, 41; 37 μ, 48; 38 μ, 40; 39 μ, 30; 40 μ, 20; 41 μ, 15; 42 μ, 11; 43 μ, 5; 44 μ, 3; 45 μ, 1; 46 μ, 1. From the 2 sets of measurements averages of 44 μ and 35 μ have been computed for diameter of oogonium and diameter of oospore, respectively.

Oospores of correct internal structure, taken from maize-meal-agar cultures that with some protection against excessive desiccation had been stored for 2 or even 3 years, were repeatedly found capable of germination. To be sure, during my earlier experience with the species, difficulty was at times encountered when oospores of unimpeachable organization, taken from cultures only a few months old, failed to germinate, or to show any other sign of continued life. The secret of such puzzling failure was eventually found to lie in the thermal requirements of *P. polymastum;* its oospores being unable to germinate and its vegetative mycelium being killed outright at the high summer temperatures often prevailing for somewhat extended periods in Washington, D. C. Recourse to artificial cooling during the summer months showed that in oospores of this as of related forms correct internal structure always betokens capability of germination.

PYTHIUM MEGALACANTHUM DE BARY SENSU BUISMAN

A fungus more impressive in its dimensions than *Pythium polymastum* was made known by Buisman (3) in 1927 in a paper devoted to root rots affecting various economic plants in The Netherlands. Its spiny subspherical oogonia had been first observed by her during the summer of the previous year, on examining roots of some flax (*Linum usitatissimum* L.) plants from Groningen affected with typical "vlasbrand," and again later in the same season, on examining flax roots from Friesland. The phycomycete was readily isolated and grown in pure culture. When mycelium from a pure culture was applied to healthy roots of flax seedlings grown in nutrient solution, softening of the main root ensued after several days. Subsequently, as the infection advanced into the lateral roots, these became glassy and limp. Roots of plants artificially inoculated showed in their tissues very numerous large spiny oogonia, which bodies reminded Buisman of the illustrations of the oogonia of *P. megalacanthum* given by de Bary. The phycomycete from flax roots seemed to her to correspond very well with the description submitted by de Bary for that species. Although she recognized that the oogonia of her fungus, ranging in diameter from approximately 30 to 70 μ, often exceeded the measurements (36 to 45 μ) given by de Bary, she held that, owing to wide variability in the structures concerned, the differences noted were of no importance. Her failure to obtain zoosporangia she held might come from refractoriness of the particular strain represented in her single culture. In fine, she identified the fungus in flax roots as *P. megalacanthum.*

In 1928 van der Meer (15) published the results of successful inoculation experiments, which not only fully corroborated Buisman's discoveries concerning the causal relationship of the large spiny fungus to "vlasbrand," but which, in addition, indicated this fungus to be the sole primary parasite responsible for the disease. Because of failure to obtain typical symptoms of the disease in plants artificially inoculated with *Thielavia basicola* (B. & Br.) Zopf or with *Asterocystis radicis* de Wild., she denied the primary pathogenicity of these 2 organisms, and held them capable only of slightly accentuating the destructive action of *Pythium megalacanthum.* Extensive investigations reported by Diddens (4) 3 years later likewise revealed the spiny oomycete as the sole cause of true "vlasbrand," though conceding some feeble parasitism to *T. basicola* in the causation of a slight root rot. Active parasitism, incidentally, was attributed by Diddens also to *P. debaryanum* Hesse and *P. irregulare* Buis., these fungi, like *P. megalacanthum,* having been found by her capable of causing serious root rot in water cultures, even if in pot cultures they caused only a kind of damping-off quite different from the typical "vlasbrand" there brought on by the spiny form alone.

Although van der Meer's conclusion as to *Pythium megalacanthum* being solely responsible for flax scorch would thus seem to have been amply corroborated by Diddens, it was nevertheless very soon disputed by Marchal (12, 13, 14), who found that in Belgium in 1930 flax plants with typical "brûlure" contained none of the spiny oogonia distinctive of *P. megalacanthum,* but consistently revealed in their affected roots numerous resting spores characteristic of *Asterocystis radicis.* Accordingly, the Belgian investigator, who had set forth the latter organism as the cause of "brûlure" in 1901, still continued 3 decades later to regard it as responsible for the disease in Flanders. He was inclined to believe, furthermore, that flax scorch is a pathological condition, which, like "damping off," might well be brought about by different root parasites in different regions,—in The Netherlands, preponderantly by *P. megalacanthum;* in Flanders, mainly by *A. radicis;* elsewhere, perhaps by species of *Thielavia* and *Hypochnus.* A similar view of the disease as a complex one, seems, indeed, to have been entertained previously by Buisman and apparently underlay also Schilling's account (20) of "Flachsbrand," which appeared in 1928. Recently, in a Belgian publication, Vanderwalle and van den Bruel (24) have characterized *P. megalacanthum* as only a saprophyte occurring on dead plants, without giving any grounds why they considered erroneous the body of evidence submitted by the several Dutch investigators in favor of its causal connection with flax scorch.

Regardless of its disputed pathogenic relationship, the flax-root fungus, a culture of which was received from the Centraalbureau voor Schimmelcultures, is one of the most striking of oomycetes. Growing on maize-meal agar its mycelium, whether examined with the naked eye or under a microscope, shows a general resemblance to the mycelium of *Pythium mastophorum* and of *P. polymastum.* The hyphae, mostly 3 to 7 µ in width, pursue somewhat random courses through the substratum, giving off branches rather sparingly and at rather wide angles. Aerial development, for the most part, is meager, resulting usually in a scant arachnoid covering, or in scattered fleecy wisps. Appressoria of the sickle-shape type (Fig. 7, A) are produced here and there

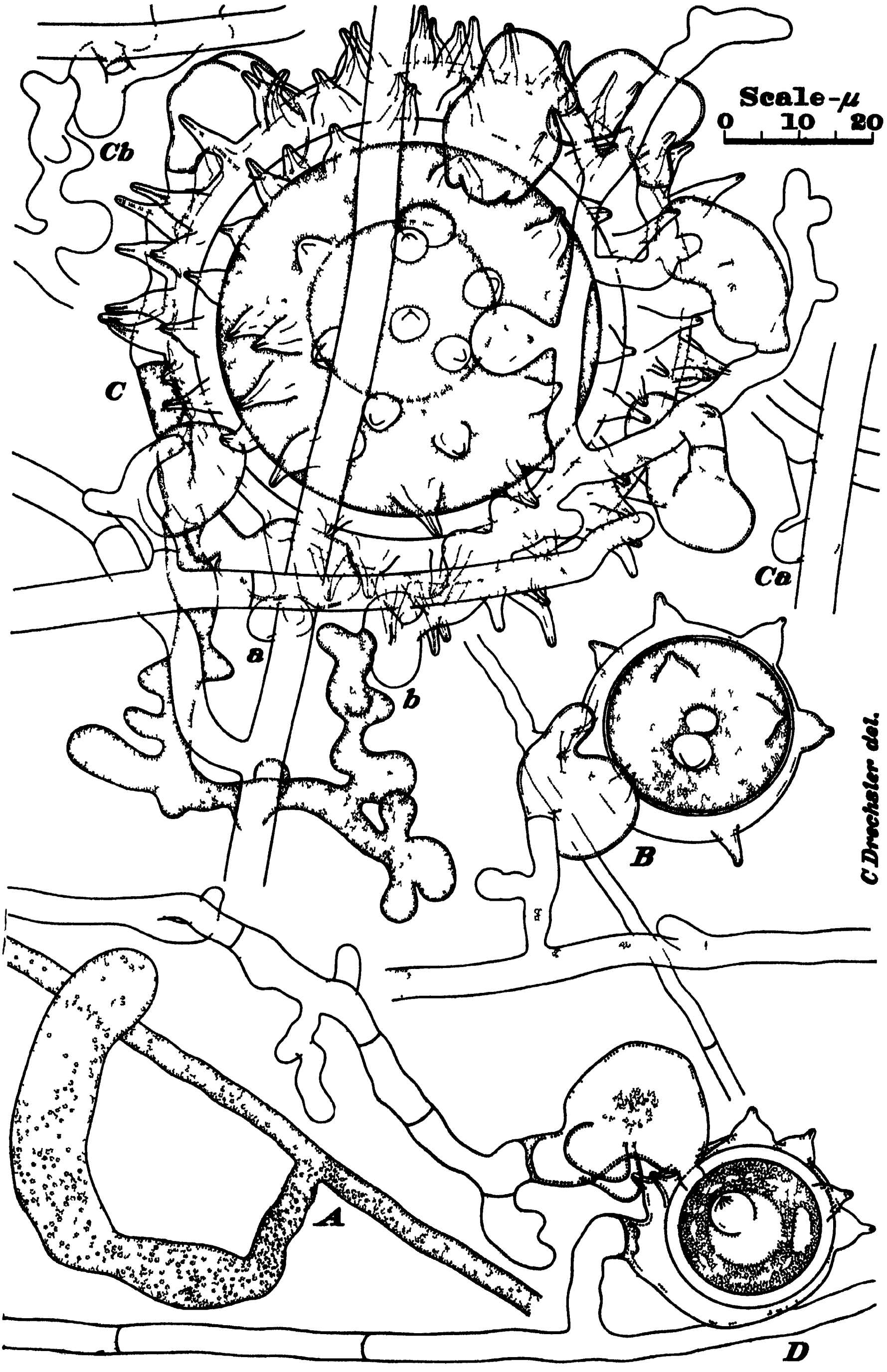

FIG 7 The flax root fungus, drawn from maize meal agar cultures, × 1000 A Appressorium. B Small immature sexual unit C Large mature sexual unit, attachment of oogonial stalk, a, and of antheridial hypha, b, being shown also separately. D. Small mature sexual unit, about 60 days old.

in contact with the hard surface of the container, where they are borne terminally on short lateral branches given off by the main hyphae. As their functional frustration sometimes leads to repetitional development, connected systems of curved elements are to be seen, though in lesser numbers than in some more familiar related forms.

After a mycelium of the fungus has been growing for 3 or 4 days it begins to produce sexual reproductive structures. The oogonia make their appearance as smooth globose bodies, for the most part borne terminally on straight or slightly curved lateral stalks usually between 5 and 20 μ long (Fig. 8, A, C, D), though some supporting stalks of more pronounced curvature may attain lengths of approximately 30 or 40 μ (Fig. 8, B; 9, A, a). The beginnings of a male complement, arising commonly from a hypha without close mycelial connection with the oogonial thread, soon become applied to the oogonium or to the hyphal elements supporting it. Sometimes the young female organ becomes inwrapped by a branching filament destined to bear one or several antheridia (Fig. 8, A). However, on the whole, contact of the growing oogonium itself with the young male structures would seem less extensive than the contact of the male parts with the oogonial stalk (Fig. 8, B, C) and an adjacent portion of parent filament (Fig. 8, D). Lobulate excrescences and diverticulations are often present on the antheridia or on the antheridial hyphae to promote intrication of male and female elements.

On attaining approximately its definitive size, the oogonium puts forth protuberances that, like the homologous modifications in *Pythium mastophorum* and *P. polymastum*, are often hemispherical or conical (Fig. 7, C; 9, A, a; B, a; 10, A, a; B, a) or somewhat mammiform (Fig. 7, B, D), not to mention the more extravagant variations in shape frequently associated with eventual degeneration of the oogonial contents. In units of sexual apparatus having normal development the protuberances measure from 1.5 to 12 μ (average about 7 μ) in length, and from 2 to 8 μ (average about 4.7 μ) in basal width; the walls surrounding them measuring 0.6 to 1.8 μ in thickness at the base to become often rather markedly thinner toward the apex. The number of protuberances visible in the upper and equatorial aspects of an oogonium varies from 5 in small specimens to about 110 in large specimens, the average lying perhaps between 55 and 60. A fairly extensive portion of the oogonial wall adjacent to the supporting stalk and representing, in most instances, from one-twelfth to one-sixth of the subspherical envelope, remains free of protuberances.

The sculptured oogonium is delimited by deposition of a thick septum usually at a distance of several microns from the large spherical part, thereby including in the female organ, as it were, an adjacent portion of its stalk. Sometimes, indeed, the delimiting cross-wall may be placed at the very origin of the stalk, so that the oogonium comes to be sessile on the parent filament (Fig. 10, B, a).

Fertilization is accomplished sometimes by a single antheridium (Fig. 7, B, D; 9, A, a; B, a; 10, B, a), but very frequently 2 (Fig. 10, A, a) or 3

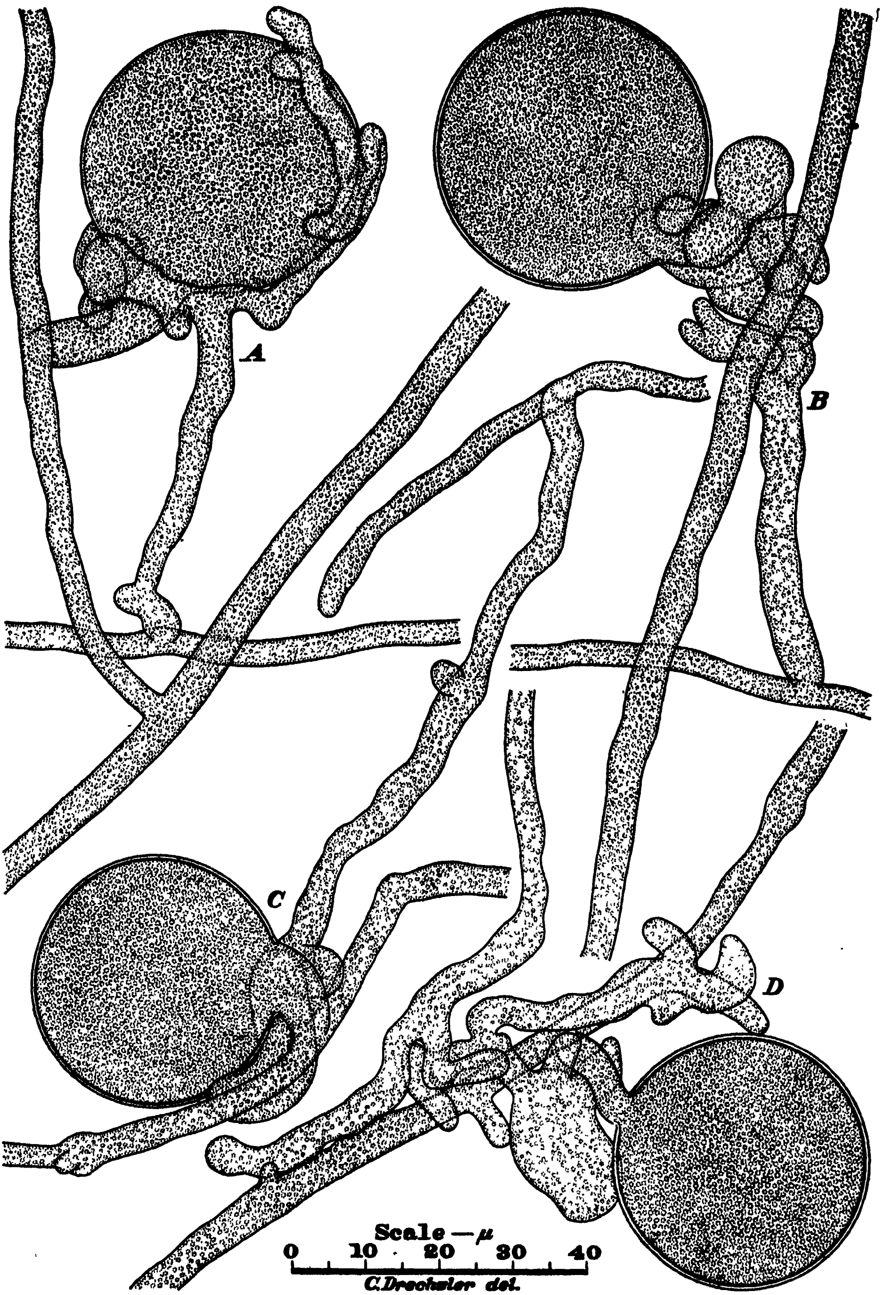

FIG. 8. Young units of sexual apparatus of the flax-root fungus, *Pythium megalacanthum* de Bary *sensu* Buisman, formed in a maize-meal-agar culture, and, with the aid of a camera lucida, drawn to a uniform magnification; × 1000 throughout. A. Unit in which the growing oogonium is being more or less enveloped by a branching antheridial filament, before any male organ becomes distinguishable. B, C, D. Units in each of which a young antheridium became recognizable as a terminal enlargement of an antheridial branch; each unit showing moderate intrication of male and female parts.

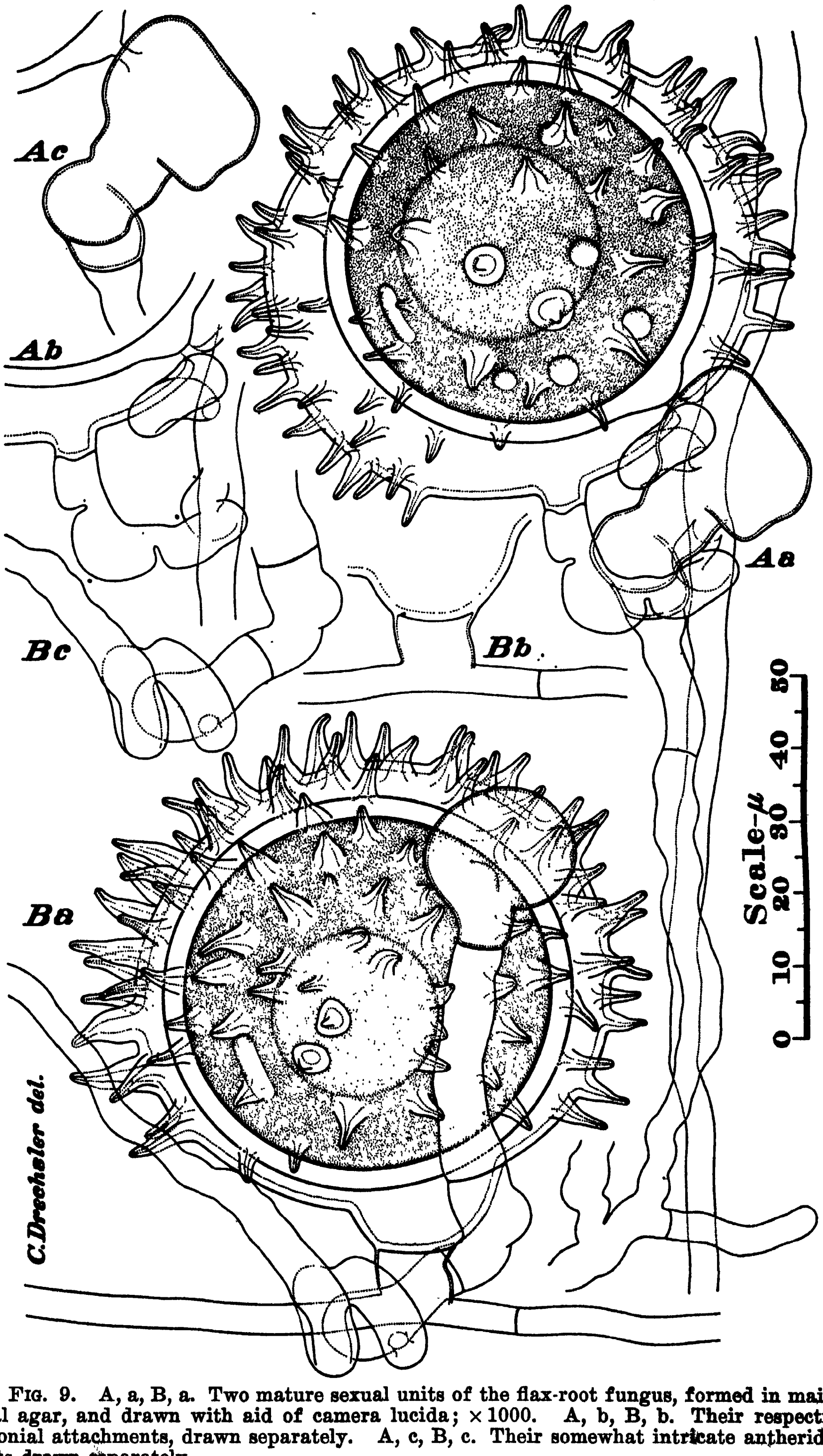

FIG. 9. A, a, B, a. Two mature sexual units of the flax-root fungus, formed in maize-meal agar, and drawn with aid of camera lucida; × 1000. A, b, B, b. Their respective oogonial attachments, drawn separately. A, c, B, c. Their somewhat intricate antheridial parts drawn separately.

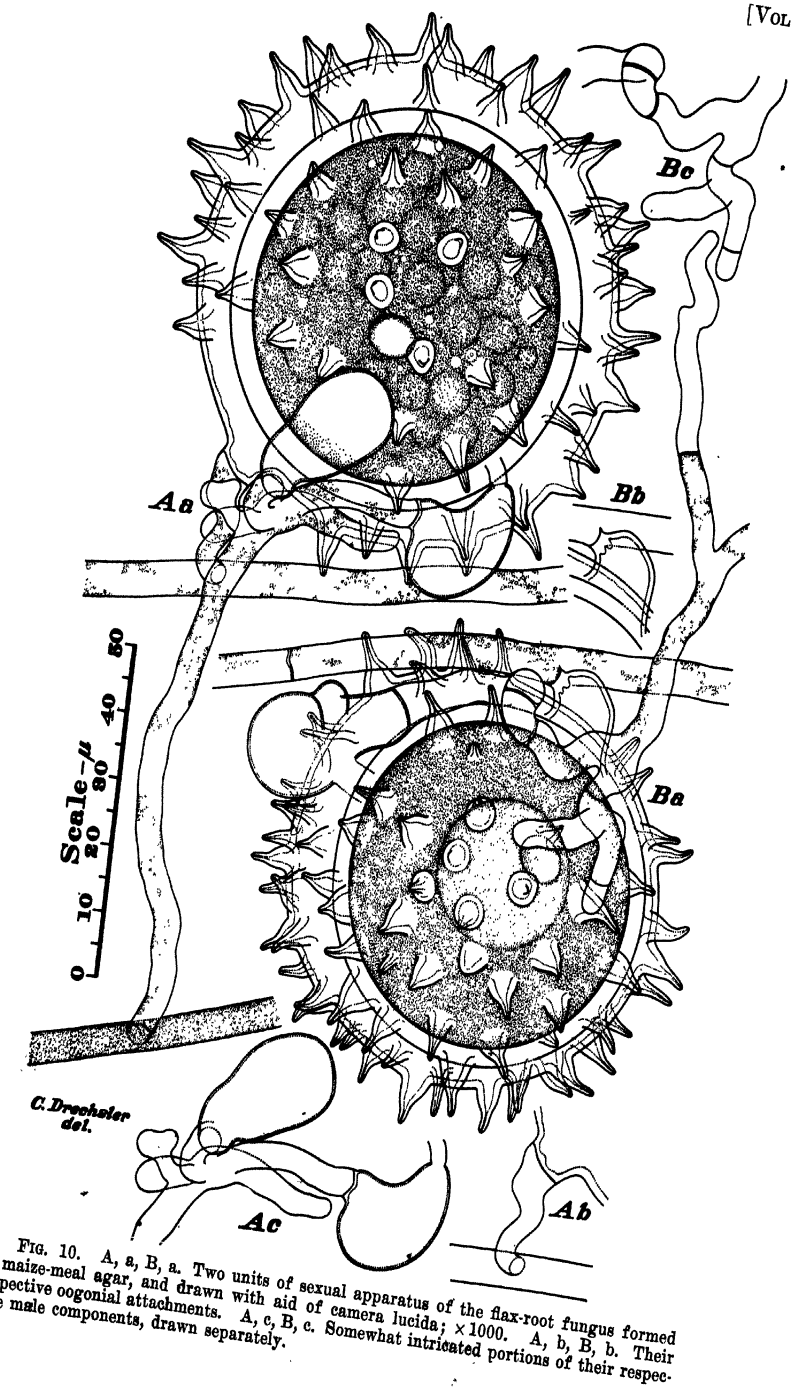

FIG. 10. A, a, B, a. Two units of sexual apparatus of the flax-root fungus formed in maize-meal agar, and drawn with aid of camera lucida; ×1000. A, b, B, b. Their respective oogonial attachments. A, c, B, c. Somewhat intricated portions of their respective male components, drawn separately.

antheridia may participate in the process, and as many as 7 have been found present (Fig. 7, C). Plural male organs appear in most cases to be supplied from a single mycelial filament. In shape the antheridia show considerable variety, swollen clavate and broadly saccate types predominating. They measure usually 18 to 40 μ (average approximately 27 μ) in length and 9 to 18 μ (average approximately 14 μ) in width. Transfer of antheridial contents into the oogonium is accomplished by means of a fertilization tube measuring 2 to 5.5 μ in length and 2 to 5 μ in diameter.

Development subsequent to fecundation is marked by the same sequence of changes as in most congeneric forms. After rounding up into a spherical shape (Fig. 7, B) the fertilized protoplast surrounds itself with a smooth wall (Fig. 10, A, a) 1 to 4.5 μ, mostly 2 to 4 μ (average 3 μ) in thickness. At early maturity the oospore, colorless or slightly yellowish, reveals a single reserve globule, 10 to 30 μ (average 23 μ) in diameter, which is surrounded by a parietal granular layer in which is imbedded a single refringent body, subspherical or strongly flattened (Fig. 7, C, D; 9, B, a; 10, B, a). Oospores in cultures several weeks or several months old show additional maturation by a perceptibly larger reserve globule, by a more coarsely granular structure of the parietal layer, and often by the presence of plural refringent bodies (Fig. 9, A, a).

Buisman noted that oogonia produced in cultures of the flax-root fungus were often empty, owing to degeneration of their protoplasmic contents. And, indeed, when the fungus is grown on potato agar or on agar media prepared with filtered maize-meal decoction, oogonial degeneration often takes place in such measure that only a few of the sexual units give rise to good oospores. Addition of a considerable quantity of very finely ground maize meal to an agar medium often brings about marked improvement, with the result that fully half of the sexual apparatus may at maturity contain oospores of correct internal structure. Partial degeneration, accompanied by endogenous development of smaller, secondary oogonia that then bear correspondingly smaller oospores, occurs also in cultures of this species. However, only oogonia of primary origin, each containing an oospore of irreproachable internal organization without any intervention of secondary development, were selected in making measurements intended for comparison with measurements of allied forms. Four hundred specimens, otherwise taken at random in maize-meal-agar cultures of more than ordinary merit with respect to normality of sexual development, gave a distribution of values for oogonial diameter, expressed to the nearest micron, as follows: 25 μ, 3; 27 μ, 1; 29 μ, 1; 30 μ, 1; 31 μ, 3; 32 μ, 4; 33 μ, 1; 34 μ, 3; 35 μ, 3; 36 μ, 3; 37 μ, 5; 38 μ, 2; 39 μ, 1; 40 μ, 4; 41 μ, 1; 42 μ, 1; 43 μ, 3; 44 μ, 4; 45 μ, 1; 46 μ, 1; 47 μ, 3; 49 μ, 1; 50 μ, 2; 51 μ, 4; 52 μ, 6; 53 μ, 6; 54 μ, 5; 55 μ, 10; 56 μ, 10; 57 μ, 14; 58 μ, 13; 59 μ, 33; 60 μ, 31; 61 μ, 28; 62 μ, 27; 63 μ, 24; 64 μ, 32; 65 μ, 25; 66 μ, 13; 67 μ, 14; 68 μ, 10; 69 μ, 10; 70 μ, 6; 71 μ, 5; 72 μ, 1; 73 μ, 5; 74 μ, 2; 75 μ, 5; 76 μ, 1; 77 μ, 3; 78 μ, 1; 79 μ, 2; 81 μ, 1; 82 μ, 1. The 400 oospores contained within these oogonia gave values for diameter, expressed to the nearest micron, with distribution as follows: 20 μ, 2; 21 μ, 2; 22 μ, 1; 23 μ, 3; 24 μ, 2; 25 μ, 1; 26 μ, 5; 27 μ, 4; 28 μ, 3; 29 μ, 5; 30 μ, 1; 31 μ, 4; 32 μ, 3; 33 μ, 9; 34 μ, 2; 35 μ, 2; 36 μ, 1; 37 μ, 5; 38 μ, 2; 39 μ, 3; 40 μ, 2; 41 μ, 8; 42 μ, 8; 43 μ, 8; 44 μ, 10; 45 μ, 9; 46 μ, 19; 47 μ, 20; 48 μ, 35; 49 μ, 32; 50 μ, 20; 51 μ, 35; 52 μ, 25; 53 μ, 22; 54 μ, 23; 55 μ, 12; 56 μ, 11; 57 μ, 10; 58 μ, 6; 59 μ, 9; 60 μ, 6; 61 μ, 3; 62 μ, 2; 63 μ, 1; 64 μ, 1; 65 μ, 2; 66 μ, 1. From the 2 sets of values were computed averages of 59.1 μ and 47.5 μ for diameter of oogonium and diameter of oospore, respectively.

Such attempts as I have made to induce the development of zoosporangia through careful irrigation of slabs removed from vigorous agar plate cultures of the flax-root fungus, failed like the earlier efforts of Buisman and Diddens directed to a similar end. As Diddens well pointed out, absence of knowledge concerning a zoosporangial phase in the life history of the fungus renders inadvisable any change in assignment from that originally made by Buisman; though, at the same time, Buisman's identification is left with much less factual support than might be desired. It seems worthy of emphasis that in de Bary's account of his *Pythium megalacanthum* is described an asexual reproductive phase that, besides displaying specific characters of ordinary distinctiveness in the shape, position, and proliferous development of the sporangia, reveals a character of extraordinary distinctiveness in the unusually large size of the encysted zoospores. Until swimming spores of comparable size, and sporangia of proliferous tendency, have been demonstrated in the fungus under consideration, its identification with de Bary's species must be regarded as very uncertain. The reflection may be permitted that should the dimensional proportionality between oospores, on the one hand, and zoospores on the other, that is evident in *P. mastophorum* and *P. polymastum,* extend to the flax-root fungus, the zoospores of that fungus, if such bodies be indeed existent, would fulfill approximately the requirements of size indicated in de Bary's account. The fact that instances of sporangial proliferation have not yet been observed in either *P. mastophorum* or *P. polymastum* argues somewhat against a likelihood of such development in an intimately related form; yet in view more especially of the frequently terminal position of the sporangium in the 2 species named, proliferation in these species and in others closely related to them would at least not seem wholly precluded on morphological grounds.

Comparison of the sexual stage produced by the flax-root fungus in maize-meal agar cultures, with the sexual stage described for *Pythium megalacanthum* by de Bary, discloses neither such convincing correspondence in structural and developmental details as would confirm a supposition of identity, nor such out-and-out disagreement as would definitely imply separateness. In *P. magalacanthum,* according to de Bary, the mycelial connection between an oogonium and the antheridium fertilizing it, is never a close one; and certainly in the flax-root fungus as well as in *P. mastophorum* and *P. polymastum,* the male organ likewise is borne on a branch only distantly connected with the oogonial filament. A marked difference is apparent, however, in respect to the developmental stage of the oogonia at which the antheridia are applied to them. Referring to the oogonia of *P. megalacanthum,* de Bary (1) wrote: "Haben dieselben ihre volle Grösse erreicht, so treten an die meisten auch Antheridien heran." In the flax-root fungus, by contrast, and, as has been noted, also in *P. mastophorum* and *P. polymastum,* the antheridia develop more or less simultaneously with the oogonia after contact of the opposite sexual elements or of the supporting hyphae has been established at an early stage. Concerning the characteristic intrication of parts, frequently, even if not constantly, evident in sexual apparatus of the 3 mamelonated fungi herein discussed, de Bary made no statement.

The oogonial protuberances of *Pythium megalacanthum* were set forth in the original accounts of that species as being of generally conical shape, from 6 to 9 μ in length, mostly broad at the base, straight or slightly curved, and fairly acute or more bluntly rounded at the apex. Approximate agreement with regard to these details is readily apparent in numerous oogonia of the flax-root fungus. If, as Diddens correctly pointed out, the spines here often attain lengths exceeding 9 μ (Fig. 7, C), it is equally true that frequently they fail to attain a length of 6 μ (Fig. 9, B). In de Bary's accounts, however, no allusion is made to dome-shaped and to mammiform variations of the oogonial protuberances, or to any conspicuous thickness of wall especially in the basal portion of the individual spine, or to any marked attenuation of the lumen in the frequently prolonged apical portion,—particulars in which are conveniently displayed the insignia of interrelationship distinguishing the small group that includes *P. mastophorum, P. polymastum* and the flax-root fungus as its known members. Of course, de Bary's failure to record such further details cannot be considered conclusive evidence that they were alien to his fungus, for often they are not expressed well in material kept more or less submerged in water.

Moreover, since species of *Pythium* are somewhat inclined to produce smaller oogonia and smaller oospores under aquatic conditions than in fairly dry, solid substrata, one should be prepared to allow for substantial differences between the measurements of these bodies submitted by earlier authors, and homologous masurements taken from firm agar cultures. Nevertheless the range in values for oogonial diameter in *P. megalacanthum* given by de Bary, 36 to 45 μ, and his approximate value for diameter of oospore, 27 μ, indicate main dimensions so much smaller than those of the flax-root fungus that the differences could be dismissed as of no importance only if satisfactory agreement prevailed with respect to most other features. Assuredly in size of oogonium and of oospore both *P. mastophorum* and *P. polymastum* conform much more closely to de Bary's description of *P. megalacanthum* than does the flax-root fungus. It seems worthy of mention, too, that the single figure of de Bary's showing an oognium of *P. megalacanthum* with a mature oospore (2, Plate 5, Fig. 12), reveals a reserve globule larger in proportion to the oospore, and a parietal layer correspondingly narrower in proportion to the oospore, than is usual in the flax-root fungus, or, for that matter, in *P. mastophorum* and *P. polymastum*. Finally, de Bary neither mentions in his text nor illustrates in his figures any marked inequality in the distribution of spines over the oogonia of *P. megalacanthum;* whereas, in the flax-root fungus, protuberances are generally lacking in a fairly extensive region of the oogonium, adjacent to or surrounding the oogonial stalk.

A few years ago I (8) briefly recorded my having observed large oogonia with somewhat mastoid, thick-walled protuberances in the tissues of discolored roots of potted cineraria (*Senecio cruentus* DC.) plants from a greenhouse near Alexandria, Virginia. Undoubtedly these oogonia belonged to a species of *Pythium* intimately related to *P. mastophorum, P. polymastum,* and the flax-root fungus. A similar taxonomic relationship may with tolerable plausibility be attributed to a fungus that Nicolas and Aggéry (16) observed earlier in France in brown discolored rootlets of yellowish stunted specimens of parsley, *Petroselinum hortense* Hoffm. In the parenchyma and near the vessels of the affected rootlets, the fungus was present for the most part as spherical thick-walled "oeufs" 28 to 36 μ in diameter exclusive of their spirally arranged "épines trapues" that measured 3 to 4.7 μ both in basal width and in length. Associated with these "oeufs" were present, though in lesser quantity, some spherical thin-walled bodies designated as sporocysts. When produced on extramatrical mycelium coming from affected rootlets bathed in water, the sporocysts were found to be terminal or intercalary in position, and to measure 10.4 to 47 μ in diameter. The form and dimensions of the "oeufs" being held to approximate the form and dimensions ascribed by de Bary to the oogonia of *P. megalacanthum,* the parsley fungus was referred to that species despite a "petit difference" recognized in lesser dimensions of the "épines trapues" relative to the longish conical spines set forth by de Bary. While, perhaps, the dimensional difference in question can plausibly be dismissed on the ground that it might derive from the variability normal to fungi, there yet remains unreconciled an apparent difference in shape of the protuberances. For, although de Bary stated that the spines of his fungus were much given to variation in form, nothing in his text or in his illustrations specifically indicates a hemispherical form as having been noted among the variations. In any case hemispherical protuberances similar to those figured by Nicolas and Aggéry actually are found on many oogonia of *P. mastophorum, P. polymastum* and the flax-root fungus, not only while the organs concerned are still immature (Fig. 2, D, E; 4, R, S; 6, D, E), but also after they have reached maturity.

Of the several passages found in additional writings where the binomial *Pythium megalacanthum* was mentioned in relation to fungi encountered at first hand, some are wanting in confirmatory observational information, while others convey observational information that neither sustains the thesis that an organism conspecific with de Bary's was concerned, nor, again, in any instance encourages a presumption that some member of the mamelonated series under consideration might have been involved. From an early incidental comment of Schroeter's (21, p. 232) it appears that this writer believed he once found de Bary's organism in stems of *Veronica hederifolia* in Breslau, though, for want of details, it is not evident on what grounds the belief was entertained. Under the name *Pythium megalacanthum,* Sideris (23) much more recently published several microphotographs of sexual apparatus pertaining to a fungus presumably isolated in Hawaii from roots of the pineapple, *Ananas sativus* Schult. From the scale of magnification indicated in the legend accompanying the microphotographs, the oogonia shown would seem to measure 27 to 33 μ in diameter, exclusive of the numerous spines, about 3 to 4 μ long, borne on them. The oospores loosely contained in 2 of the spiny envelopes give measurements for diameter of approximately 24 and 27 μ, and seem to be provided with smooth walls despite a characterization of the oospores of the species in the author's analytical key as "large, terminal, with long spines," and despite, too, the inclusion of the species in a subsection supposedly distinguished by spiny oospores. According to the key the Hawaiian fungus develops epigynous antheridia whose broad saccate shape undergoes little change in consequence of fertilization. As to further details, it would appear that germination of asexual reproductive bodies takes place frequently by emission of vegetative hyphae, rarely by formation of zoospores; and that the hyphae of the fungus are irregular, the "colony faintly developed with small superficial whitish specks."

Under the name *Pythium megalacanthum,* too, was reported (11) from France in 1934 a fungus that, after having been isolated the previous year frem melon plants affected with wilt and collar-canker, had been found capable of bringing forth the original symptoms in inoculation experiments performed by Labrousse. The next year Petri (17), in Italy, recorded the isolation from infected roots of the orange, *Citrus limonum* Risso, of 2 species of *Pythium,* whereof one with spiny oogonia measuring 33 to 35 μ in diameter inclusive of the spines, 4 to 5 μ long, borne on them, was held perhaps referable to *P. megalacanthum.* According to a very recent account by Salmon and Ware (19) specimens of watercress originating from unproductive beds in Sussex, England, and bearing spores with thick spiny walls in the soft parts of the collapsed main stem, were reported by Cook to have yielded cultures of only a single fungus,—a species of *Pythium* that would probably prove to be *P. megalacanthum.* Very recently, also, van Poeteren (18) reported that at Zouderwoude, The Netherlands, active decay in cuttings of various ornamentals, including members of the genera *Chrysanthemum, Pelargonium* and *Primula,* was found attributable to *P. megalacanthum;* his report, very regrettably, however, offering no comment as to how closely the causal agent in question might resemble the spiny flax-root fungus prominent in Dutch phytopathological literature.

In considerations touching the taxonomic affiliations of *Pythium megalacanthum,* the possibility can hardly be ignored that the fungus dealt with by de Bary may have been a form alien to the series under discussion. As was intimated earlier (9, p. 420) it may perhaps have been a form more closely related to *P. anandrum,* which, like de Bary's species, combines spiny oogonia with proliferous sporangia,—a combination not frequent in the genus *Pythium,* and hitherto not demonstrated for any of the 3 species distinguished by thick-walled oogonial ornamentation. The same infrequent union of morphological features is found in an interesting fungus recently described by Shanor (22) as *Phytophthora stellata.* Though the antheridia of this fungus usually have a close mycelial connection with the oogonia fertilized by them, the mere presence of these organs provides a detail wherein the parallelism with de Bary's species is borne out better than in the parthenogenetic *P. anandrum.* Other such details are evident in the conformation of the acutely pointed, broad-based, conical spines figured by Shanor, and in the usual development of a distinct evacuation tube by the sporangia of *P. stellata* preliminary to discharge. On the other hand, it must be admitted that in diameter of oogonium (15.5 to 22.4 μ) and length of oogonial spines (up to 3.6 μ) *P. stellata* shows little—less than *P. anandrum*—of the dimensional impressiveness that led de Bary to characterize his *P. megalacanthum* as "eine sehr stattliche Form."

SUMMARY

The diagnoses of *Pythium mastophorum* and *P. polymastum* published earlier are supplemented by an illustrated discussion of the morphology and development of these fungi. From the general similarity in habit and structural detail revealed by them, the 2 species are considered to be intimately related to each other. In the same intimate relationship is rather obviously embraced also the extraordinarily large oomycete that under the binomial *P. megalacanthum* has been made known by several Dutch investigators as causing flax scorch in The Netherlands. While agreeing with de Bary's original description of *P. megalacanthum* in some particulars, the sexual apparatus of the flax-root fungus disagrees with that description in other and no less important particulars. Not any of the later literature wherein de Bary's binomial is mentioned in connection with a plant disease can be held to contain very convincing evidence that the fungus to which this binomial was originally attached has actually been rediscovered in recent times. The true *P. megalacanthum* may even prove to be alien to the *mastophorum* series herein treated, perhaps having closer affiliations with such spiny proliferous forms as *Pythium anandrum* and *Phytophthora stellata.*

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RELATIVE IMPORTANCE AND SEASONAL PREVALENCE OF WOOD-STAINING FUNGI IN THE SOUTHERN STATES

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INTRODUCTION

Several investigators (1, 3, 5, 6) have described or listed fungi associated with discolored sapwood of logs and lumber of many tree species in the United States. There is, however, nothing in the literature to indicate the

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relative importance of the different staining fungi at different times of year. It was thought that any seasonal variations might be most pronounced in the Southern States, where conditions are favorable for stain development during the greater part of the year. The present investigations were undertaken during 1937 and 1938 in Louisiana, Mississippi, and Georgia to determine the fungus floras in stained wood of different tree species, in logs and lumber, and in widely separated localities; and, more particularly, to determine the effect of season on such floras. There was need for further basic data on these subjects before either fundamental or certain of the practical phases of the stain problem could be most intelligently studied. Such information on stain floras would be helpful in explaining any importance of log stain in seasoning lumber, and the differential effectiveness of certain chemical control treatments on pine and hardwoods. It should also be helpful in determining whether such stain investigations as control tests and dissemination studies must be widely distributed geographically and seasonally and whether tests must include many tree species because of differences in stain floras in different localities, seasons, or wood species.

METHODS

Material stained in the log, referred to as "log stain," was secured in the mills at the time the logs were sawed into lumber. Cultures usually were made within 24 hours of securing the specimens by transplanting pieces from the interior of the stained wood to malt-agar plates.

To determine the fungi associated with stain developing in lumber during seasoning, referred to as "lumber stain," freshly sawed boards were placed in or under regular air-seasoning piles, where they would not dry too rapidly and thus fail to stain. Care was taken to secure boards from logs showing no log stain and cut several inches from the log end in order to eliminate any infection that might have been present in the log. After the boards had been in the seasoning yard 2 to 4 weeks, depending on temperature conditions, isolations were made from the interior of the stained areas. At Savannah, small blocks of fresh wood were placed for 24 to 36 hours in seasoning piles and then incubated for 10 days in sterile jars before culturing.

The wood species used were those commonly cut for lumber in the Southern States, viz., longleaf pine (*Pinus palustris* Mill.), loblolly pine (*P. taeda* L.), slash pine (*P. caribaea* Morelet), shortleaf pine (*P. echinata* Mill.), red gum (*Liquidambar styraciflua* L.), yellow poplar (*Liriodendron tulipifera* L.), sycamore (*Platanus occidentalis* L.), oak (*Quercus* spp.), tupelo (*Nyssa* sp.), beech (*Fagus grandifolia* Ehrh.), magnolia (*Magnolia* sp.), and hickory (*Hicoria* sp.). Longleaf pine and red gum predominated.

Samples were collected chiefly at mills located in Natalbany and Bogalusa in southeastern Louisiana during the period from April 1937 to March 1938. At these places isolations were attempted at monthly or more frequent intervals. Supplementary studies were made at Clarks, in northern Louisiana, at Bruce, in northern Mississippi, and at Savannah, Georgia, to get an indication of the staining flora at the northern and eastern extremes of the southern pine belt.

Since a uniform number of samples could not be cultured for each period, the results are presented as the percentages of samples cultured. In the case of log stain the number of samples does not necessarily mean the number of logs from which they came, as it was not always possible to separate samples by logs. The number of lumber-stain samples refers to the number of individual stain areas developing. Frequently as many as 6 or 8 samples came from one board. When the whole board was stained to such an extent that individual stain areas were not discernible, it was treated as one sample.

It is realized that the complete floras occurring in lumber stored for 2 to 12 months, as is common in air-seasoning practice, may not have been secured in the short periods of exposure used, but it is thought that the important staining fungi of freshly sawed lumber were secured. All the chief staining fungi listed for the Southern States by Davidson (1) were isolated. Greater exposure periods than those adopted proved impracticable because of the interference of nonstaining organisms. Since more than one fungus were usually isolated from a specimen, it is possible that some of the staining fungi were obscured by other organisms. Experience indicates that the important staining fungi, at least, usually can be detected in combinations with one another and most of the other organisms encountered and that the error due to obscuring would affect the data presented only in a minor way.

As is usual in the South, lumber to be air-seasoned at the mills visited is chemically treated to prevent staining. But all the material used in this study, except a few samples treated for transit only, was untreated by a chemical solution. Since the chemical treatment affected only the surface and, since isolations were made from the interior of the wood, the treatment presumably did not influence the data. The data presented may include species of fungi that are of little or no importance on chemically treated wood. Work in progress indicates, however, that the important fungi in treated wood are among those listed as important in this paper.

To help evaluate the importance of the fungi isolated, simple inoculation tests were made. Freshly sawed sapwood blocks of gum and pine were surface-sterilized in boiling water, inoculated with isolates of many of the species of the fungi isolated, and stored in sterile jars under room conditions (25° to 30° C.). The rate of spread and the intensity of color produced in the test blocks, along with frequency of isolation and observed staining capacity in the field, were used in determining the relative importance of the various staining fungi.

FUNGI ISOLATED FROM STAINED WOOD

Fungi Isolated from Pines

Important Staining Fungi. Based on the frequency of isolation and staining ability, the most important fungi found in pine logs and lumber were: *Ceratostomella pilifera* (Fr.) Winter, *C. ips* Rumbold, *Diplodia natalensis* Evans, and *D.* sp. (undetermined). The *Ceratostomellas* have been adequately described in previous literature (1, 3, 6).

Diplodia natalensis Evans is morphologically indistinguishable from *D. gossypina* Cooke on cotton (2, 9) and other crops in southern United States. Both species are now referred to the same perfect stage, *Physalospora rhodina* (Berk. and Curt.) Cooke (10). The cotton *Diplodia* has been described as usually having a lower temperature range than the citrus *Diplodia, D. natalensis* (9); but isolates tested from cotton, pear, and tung from Louisiana and Mississippi seem to be mostly of the high-temperature group (growing rapidly at 35° to 37° C.), as are those from lumber.

Diplodia natalensis is easily distinguished from other staining fungi in culture by its rapid growth (Table 5) and by the production of characteristic pycnospores in 3 or 4 weeks at room temperatures. The first spores formed are unicellular, hyaline, averaging 13.1 × 26.9 μ (44 spores), while spores formed later are bicellular, dark brown, show longitudinal striations in the spore wall, and are commonly[2] 12.0 to 14.4 × 24.0 to 28.8 μ averaging 13.5 × 26.9 μ (226 spores).

The fourth fungus of importance as a stainer on pines, tentatively referred to *Diplodia*, has never been isolated from hardwoods. It is, apparently, a hitherto unreported staining fungus, but undoubtedly one of importance in pine at those mills visited by the writer. On malt agar this *Diplodia* grows moderately fast (15 to 20 mm. in 3 days at 28° to 30° C.), forming a dense, black, and often hard mat that, with age, develops a metallic luster at the surface and a filamentose margin (Fig. 1). Aerial mycelium

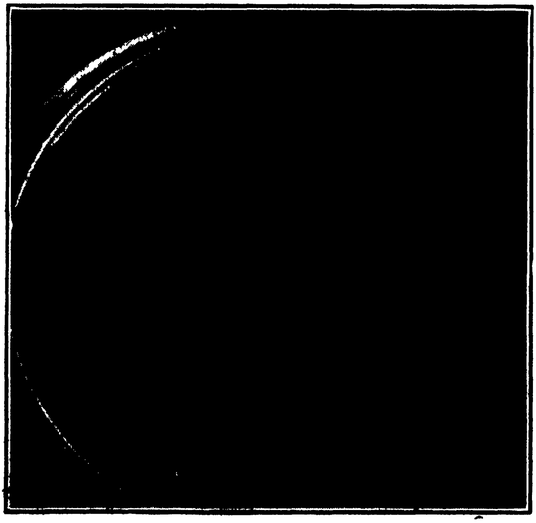

FIG. 1. Eleven-day-old culture of *Diplodia* sp. on malt agar at room temperature, 25° to 30° C.

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[2] The common range was obtained from the measurements remaining after the smallest and the largest tenth had been discarded.

may be entirely lacking and usually is limited to tufts varying from gray to nearly black, which can easily be rubbed off leaving the lustrous surface of the main mat. Partly imbedded in the mat or in the aerial mycelium, pycnidium-like masses form, but few fertile pycnidia have been found and those only after 3 to 8 weeks at room temperature.

Pycnidia are black, carbonaceous, single or in small groups, inverted clavate or globose with a distinct rostrum, ostiolate, commonly 202 to 386 µ high × 151 to 269 µ wide, averaging 271 × 208 µ (8 pycnidia), and smooth. Conidiophores are simple, hyaline, 20 to 32 × 2 µ. Spores are mostly straight, at first unicellular and hyaline but becoming distinctly yellow before maturity, and, finally, a golden brown, as the septae form. No large masses of hyaline, unicellular spores are formed, as in *Diplodia natalensis.* Mature spores are 0- to 3-septate, mostly 1-septate, granular, nonstriated, sometimes slightly clavate and tapering toward the base, which may be truncate, and are commonly 12 to 16 × 32.4 to 42 µ, averaging 13.7 × 37.3 µ (127 spores from 8 isolates). The fungus does not grow at 37° C. and has not been found fruiting in nature. In fruiting characteristics this fungus closely resembles *D. megalospora* Berk. and Curt. (*Sphaeropsis ellisii* Sacc.), but in general cultural characteristics it is distinct from the *D. megalospora* previously isolated from wood (1). It is possibly a variety of *D. megalospora.*

Minor Fungi

The following staining fungi, isolated less frequently or with poor staining ability, are considered of minor importance on pines in the Southern States:

Ceratostomella obscura Davidson (1).[3] This fungus, which was isolated but twice, has light-colored mycelium.

Ceratostomella pini Münch (6). This species is of importance in other localities (4, 6) chiefly in association with *Dendroctonus* beetles, at least in the United States. The infrequent isolation of *C. pini* in this study was probably due to the general absence of *Dendroctonus frontalis* Zimm. in the test material used. This is the beetle with which *C. pini* usually is associated in southeastern United States (6). It is conceivable that *C. pini* may become of major importance when periodic outbreaks of *D. frontalis* occur. The occurrence of *C. pini* in 5 samples at Clarks, La. (Table 3) is the only reported occurrence of this fungus in lumber stain.

Ceratostomella exigua Hedgc. This fungus is unreported in literature since the original description in 1906 (3). Two isolates of a *Ceratostomella*, more nearly corresponding to *C. exigua* than any other described species, were secured at Clarks.

Ceratostomella multiannulata Hedgc. and Davidson (1). This species was commonly found fruiting on the surface of pine lumber but was seldom isolated from the interior of stained wood. Because of its light-colored mycelium and poor staining capacity in artificial inoculations, it is considered of little importance.

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[3] Descriptions of the species listed may be found in the literature citations after each species.

Ceratostomella pluriannulata Hedgc. (3). This fungus was isolated but a few times from pine.

Endoconidiophora moniliformis (Hedgc.) Davidson (1). *E. moniliformis,* which was isolated infrequently and has light-colored mycelium, is apparently of little importance on pine.

Endoconidiophora coerulescens Münch (4). This fungus was reported as an important cause of stain on conifers in Europe (4) and certainly is of importance on hardwoods in the Southern States. Although it was isolated but twice from pine in this study, pine was readily stained when artificially inoculated with hardwood strains of *E. coerulescens.*

Alternaria spp. Several species of *Alternaria* were isolated from stained pine, but were usually associated with one or more of the important staining fungi. When alone they are associated with light-colored stain.

Helminthosporium geniculatum Tracy and Earle. Although this species is a vigorous stainer, as shown by inoculation tests, it was usually not isolated in sufficient numbers to be of great importance.

Pullularia pullulans (de Bary) Berkhout (4, as *Hormonema dematioides*) and probably other species. Although commonly found in the air of mill yards, *Pullularia* was of infrequent occurrence in stained wood.

Cadophora spp. (*C. repens* Davidson, *C. brunnescens* Davidson, *and Possibly Other Species* (1)). *Cadophora* was found chiefly in association with beetle injury and with some major staining species. *Cadophora* was often isolated from old logs but seldom from freshly stained material.

Cladosporium sp. (3). Although common on the surface of logs, and to a lesser extent of lumber, *Cladosporium* seemed to be of little importance in the material used in this study. Inoculations showed it to have little ability to penetrate wood.

Leptographium sp. (1). An unidentified *Leptographium* was isolated once from pine.

Graphium rigidum (Pers.) Sacc. *and Possibly Related Species* (3). *Graphium* was not isolated in sufficient numbers from stained pine to be considered important.

A few other fungi with dark mycelium and possibly with staining ability were isolated, but usually only in small numbers. Their identification was not attempted. Some of these were probably species listed above, but, because of mixture with other organisms, were unrecognizable.

Besides these staining fungi numerous nonstaining organisms were isolated, particularly at those mills where pine logs were stored in mill ponds. Bacteria, yeasts, *Trichoderma, Fusarium, Pestalozzia funerea* Desm., wood destroyers, *Penicillium,* and a nematode (possibly *Aphelenchoides xylophilus* Steiner and Buhrer) were the most common ones isolated. On an average about 1½ nonstaining organisms were isolated from each sample.

Fungi Isolated from Hardwoods

Important Staining Fungi. Based on the frequence of isolation and staining ability, the important staining fungi found in hardwood logs and lumber were: *Endoconidiophora coerulescens* Münch (1, 4), *Diplodia natalensis* Evans, *Ceratostomella pluriannulata* Hedgc. (1, 3), and *Graphium rigidum* (Pers.) Sacc. (3). *E. coerulescens* and *D. natalensis* are probably of greatest importance because they grow more rapidly and cause a more intense stain than does *C. pluriannulata* and *G. rigidum.*

Minor Fungi. Other staining fungi less frequently isolated or of less importance were:

Endoconidiophora moniliformis (Hedgc.) Davidson (1, 3) was one of the most abundant fungi found on stained hardwoods but, if unaccompanied by other staining fungi, was associated with a light stain that fades noticeably on drying. It is of doubtful importance as a staining fungus.

Torula, resembling *T. ligniperda* (Willk.) Sacc. (8), was isolated twice and, therefore, is apparently of little importance.

Alternaria spp. were usually associated with other staining fungi and, when alone, seemed to cause but a light-colored stain.

Cladosporium sp. has been found causing surface flecking of hardwood lumber at a number of mills. Discoloration was limited largely to the surface.

Helminthosporium geniculatum Tracy and Earle is a vigorous stainer but was infrequently isolated.

At least two species of *Leptographium* were isolated. They are of infrequent occurrence.

A few isolates of unidentified species of *Graphium* were isolated.

A few unidentified isolates with dark-colored mycelium and possibly with staining ability also were encountered. Some of these may have been species listed above, but, because of mixture and other organisms, were unrecognizable.

Fewer species of nonstaining organisms were isolated from hardwoods than from pines. On hardwoods, bacteria, wood-destroyers, and *Fusarium* were the most frequently isolated nonstaining organisms.

COMPARISON OF STAINING FLORAS ISOLATED FROM PINES AND HARDWOODS

Previous work (1) showed that, in general, hardwood stain yielded species of fungi different from those obtained from pine. This also was true in the present study. Of the *Ceratostomellas* encountered in this study, only *C. pluriannulata* was a predominantly hardwood-inhabiting species, the other species being restricted almost entirely to pines. The species of *Endoconidiophora,* on the other hand, were important only in hardwoods. *Graphium,* although isolated a number of times from pines, was much more frequent in the hardwood species. The unidentified species of *Diplodia* was restricted entirely to pines, while *D. natalensis* apparently was of importance in both pines and hardwood. *Cadophora* and *Pullularia* were primarily pine-inhabiting fungi, while the other minor staining fungi, except the *Ceratostomellas* mentioned above, showed no preference for wood species.

There were no differences in floras from different pine species or from different hardwood species. Among the different hardwood species studied there were, however, differences in the amount of stain developing under similar conditions, and, apparently, these were due to differences in susceptibility to staining in general and not to differences in fungi attacking the various woods. The hardwood species most susceptible to staining and best adapted for test purposes seemed to be red gum. No difference in the susceptibility of different pine species was evident.

COMPARISON OF LOG-STAINING AND LUMBER-STAINING FLORAS

In both pines and hardwoods the staining-fungus populations of logs were, in general, the same as those of lumber, except that, from pine, *Diplodia sp.* and *Cadophora* were uniformly more frequent in logs (Tables 1 and 2; Fig. 2).

Davidson (1) had shown that the important staining fungi in lumber were also the important ones in logs. The present study shows that the similarity of staining floras in lumber and logs also holds for the less important staining fungi, many of which previously had been reported only from logs or only from lumber. Floristically speaking there seems no need to distinguish between log and lumber stains.

In pine logs *Ceratostomella ips* was nearly always associated with galleries of *Ips* beetles, while the other fungi were found largely on the log ends or debarked places. *C. ips* has been shown to be closely associated with *Ips* beetles (6), and it is assumed that its occurrence in the seasoning yard also is chiefly associated with visitations of *Ips* beetles, which, at times, are abundantly attracted to freshly sawed pine lumber (7).

STAINING FUNGI ISOLATED IN DIFFERENT LOCALITIES

With the exception of *Diplodia* sp., not *D. natalensis,* the important staining fungi isolated in southeastern and northern Louisiana, northern Mississippi, and eastern Georgia, were the same (Tables 1 to 4). The absence of *Diplodia* sp. among the fungi isolated in Georgia and of some of the minor staining fungi in Mississippi and Georgia may have been attributable to the limited culture work done in these States.

Davidson (1) did not report the undetermined species of *Diplodia* among the isolates he secured in Louisiana, Florida, and Mississippi in 1931 and 1932. Some of the mills visited by Davidson were among those where *Diplodia* sp. was important during 1937 and 1938. Therefore, one would surmise that if *Diplodia* sp. actually was absent in eastern Georgia when isolations were made in the present study, its absence might be due more to the scarcity of this species during certain years than to any influence of location.

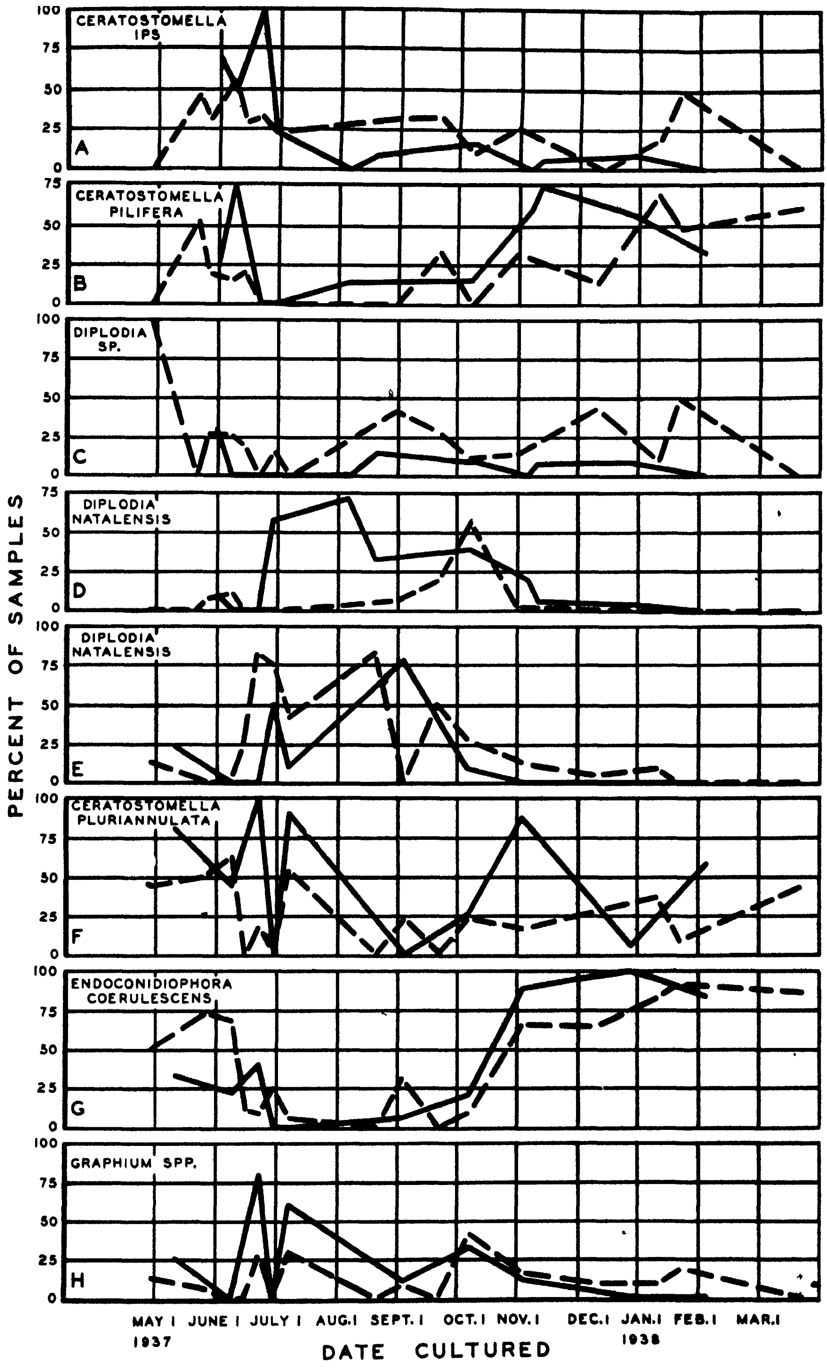

FIG. 2. Per cent of samples yielding the indicated important strain fungi on different dates in southeastern Louisiana. Solid lines indicate isolations from stained lumber and broken ones those from stained logs. A to D, from pines. E to H, from hardwoods.

TABLE 1.—*Frequency of isolation of fungi from stained pine lumber and logs in southeastern Louisiana (Natalbany and Bogalusa) on different dates*

Date and kind of stained sample	Samples cultured	Per cent of samples yielding—																			
			Ceratostomella										*Diplodia*		*Endoconidiophora*						
		Alternaria	*exigua*	*ips*	*multiannulata*	*obscura*	*pilifera*	*pini*	*plurannulata*	spp.[a]	*Cadophora*	*Cladosporium*	*natalensis*	sp.	*coerulescens*	*moniliformis*	*Graphium*	*Helminthosporium*	*Pullularia*	Dark undetermined[b]	Nonstaining[c]
Lumber:																					
June 1, 1937	30	20	3	70	0	0	27	0	3	0	0	0	10	30	0	0	0	17	0	13	67
June 7, 1937	4	25	0	50	0	0	75	0	0	0	0	0	0	0	0	0	0	0	0	0	75
June 21, 1937	4	0	0	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	100
June 28, 1937	37	0	0	24	0	0	0	0	0	8	0	0	57	0	0	0	0	0	5	43	97
Aug. 6, 1937	7	0	0	0	0	0	14	0	0	0	0	0	71	0	0	0	0	0	0	14	100
Aug. 19, 1937	22	14	0	9	0	0	14	0	0	5	0	0	32	14	0	0	0	23	0	14	36
Oct. 7, 1937	69	4	0	17	0	0	16	0	1	1	0	0	39	9	0	12	7	10	0	23	100
Nov. 6, 1937	5	0	0	0	0	0	60	0	0	0	0	0	20	0	0	0	20	0	0	0	100
Nov. 10, 1937	36	33	0	6	0	0	75	0	3	0	0	0	6	8	0	17	8	6	0	17	97
Dec. 27, 1937	34	27	0	9	9	0	56	0	3	0	0	0	3	9	3	0	0	0	0	3	79
Feb. 3, 1938	12	42	0	0	0	0	33	0	50	0	0	0	0	0	0	8	0	0	0	8	100
Total summer[d]	139	4	0	19	0	0	11	0	1	4	0	0	43	6	0	6	4	9	1	32	84
Total fall, winter, and spring[d]	121	27	1	23	2	0	53	0	7	0	0	0	6	12	1	6	3	6	0	10	84
Logs:																					
Apr. 27, 1937	2	0	0	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	50
May 20, 1937	17	6	0	47	0	6	53	0	0	0	6	0	0	0	0	12	0	0	0	12	53
May 25, 1937	15	0	0	33	0	0	20	7	0	0	20	0	7	27	0	0	0	0	0	13	100

[a] Obscured by other organisms and probably mostly the other species listed.

[b] Probably includes some of the other fungi listed, but if so they were obscured by other organisms.

[c] Includes bacteria, yeasts, and higher fungi.

[d] Summer includes isolates from June 21 to Oct. 8, inclusive; and fall, winter and spring, all other dates.

TABLE I.—(*Continued*)

Date and kind of stained sample	Samples cultured	Per cent of samples yielding—																			
			Ceratostomella										*Diplodia*		*Endoconidiophora*						
		Alternaria	*exigua*	*ips*	*multiannulata*	*obscura*	*pilifera*	*pini*	*pluriannulata*	spp.[a]	*Cadophora*	*Cladosporium*	*natalensis*	sp.	*coerulescens*	*moniliformis*	*Graphium*	*Helminthosporium*	*Pullularia*	Dark undetermined[b]	Nonstaining[c]
Logs, cont.:																					
June 7, 1937	19	5	0	58	0	0	16	11	0	5	0	0	11	26	0	5	0	0	0	0	26
June 14, 1937	10	0	0	30	0	0	20	0	0	0	10	0	0	20	0	0	10	20	0	30	50
June 21, 1937	3	0	0	33	0	0	0	0	0	0	67	0	0	0	0	0	0	0	0	33	100
June 28, 1937	13	0	0	23	0	0	0	0	0	0	0	0	0	15	0	0	0	0	0	69	100
July 6, 1937	4	0	0	25	0	0	0	0	0	0	50	0	0	0	0	0	0	0	0	75	100
Sept. 1, 1937	36	0	0	33	0	0	0	0	0	0	17	3	6	42	0	0	0	3	0	19	61
Sept. 21, 1937	21	0	0	33	0	0	33	0	0	0	5	0	19	29	0	0	0	0	0	10	86
Oct. 8, 1937	9	0	0	11	0	0	0	0	0	0	0	0	56	11	0	0	11	0	0	22	67
Oct. 30, 1937	33	6	0	27	0	0	33	0	0	3	9	0	3	15	0	0	3	0	0	42	88
Dec. 10, 1937	7	14	0	0	0	0	14	0	0	0	29	0	0	43	0	0	0	14	0	14	100
Jan. 10, 1938	10	0	0	20	10	0	70	0	0	0	0	0	0	10	0	0	0	0	0	20	100
Jan. 21, 1938	2	0	0	50	0	0	50	0	0	0	0	0	0	50	0	0	0	0	0	0	50
Mar. 22, 1938	8	13	0	0	0	0	63	0	0	0	0	0	0	0	0	0	0	0	0	25	100
Total summer[d]	86	0	0	29	0	0	8	0	0	0	12	1	12	28	0	0	1	1	0	28	77
Total fall, winter, and spring[d]	123	5	0	32	1	1	34	2	0	2	8	0	3	19	0	2	2	2	0	21	65
Lumber and logs:																					
Total summer	225	3	0	23	0	0	10	0	1	2	5	1	32	15	0	4	3	6	1	30	81
Total fall, winter, and spring	244	16	+	27	2	+	43	1	4	1	4	0	5	16	+	4	3	4	0	16	75

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[a] Obscured by other organisms and probably mostly the other species listed.

[b] Probably includes some of the other fungi listed, but if so they were obscured by other organisms.

[c] Includes bacteria, yeasts, and higher fungi.

[d] Summer includes isolates from June 21 to Oct. 8, inclusive; and fall, winter, and spring, all other dates.

TABLE 2.—*Frequency of isolation of fungi from stained hardwood lumber and logs in southeastern Louisiana (Natalbany and Bogalusa) on different dates*

Date and kind of stained sample	Samples cultured	Percent of samples yielding—												
				Ceratostomella			*Endoconidiophora*							
		Alternaria	*Cladosporium*	*pluriannulata*	spp.[a]	*Diplodia natalensis*	*coerulescens*	*moniliformis*	*Graphium*	*Helminthosporium*	*Leptographium*	*Torula*	Dark undetermined[b]	Nonstaining[c]
Lumber:														
May 9, 1937	43	7	0	81	0	23	33	63	26	16	0	0	2	63
June 7, 1937	9	0	0	44	0	0	22	78	0	0	0	0	0	67
June 21, 1937	5	0	0	100	0	0	40	60	80	0	0	0	20	100
June 28, 1937	4	0	25	0	0	50	0	0	0	0	0	0	25	100
July 6, 1937	10	0	0	90	0	10	0	20	60	0	0	0	10	100
Sept. 3, 1937	18	6	0	0	0	78	6	0	11	0	0	0	11	83
Oct. 6, 1937	35	3	0	26	0	9	20	69	32	0	0	0	0	91
Nov. 3, 1937	9	11	0	88	0	0	88	11	11	0	0	0	0	22
Dec. 27, 1937	18	0	0	6	0	0	100	0	0	0	0	0	0	83
Feb. 4, 1938	12	0	0	58	0	0	83	17	0	0	0	0	8	100
Total summer[d]	72	3	1	29	0	28	14	40	29	0	0	0	7	92
Total fall, winter, and spring[d]	91	4	0	60	0	11	57	41	13	8	0	0	2	67
Logs:														
Apr. 27, 1937	16	13	0	44	13	13	50	25	13	0	6	6	0	56
May 25, 1937	18	0	0	50	0	0	73	95	6	0	6	0	17	67
June 7, 1937	16	6	0	63	0	0	69	50	0	0	0	0	0	50
June 14, 1937	9	0	0	0	0	22	11	33	0	0	0	0	56	67
June 21, 1937	11	0	0	18	0	82	9	9	27	0	0	0	18	91

[a, b, c, and d] See footnotes to table 1.

TABLE 2—(*Continued*)

Date and kind of stained sample	Samples cultured	Percent of samples yielding—												
				Ceratostomella			*Endoconidiophora*							
		Alternaria	*Cladosporium*	*pluriannulata*	spp.^{a}	*Diplodia natalensis*	*coerulescens*	*moniliformis*	*Graphium*	*Helminthosporium*	*Leptographium*	*Torula*	Dark undetermined^{b}	Nonstaining^{c}
Logs, cont.:														
June 29, 1937	4	0	0	0	0	75	25	0	0	0	0	0	0	75
July 6, 1937	17	0	0	53	0	41	6	41	29	0	0	0	12	82
Aug. 20, 1937	6	0	0	0	0	83	0	17	0	0	17	0	0	100
Sept. 3, 1937	13	0	0	23	0	0	31	54	8	15	0	0	0	46
Sept. 21, 1937	2	0	0	0	0	50	0	50	0	0	0	0	0	100
Oct. 6, 1937	32	0	0	22	0	28	9	72	41	0	0	0	13	91
Nov. 2, 1937	31	0	0	16	0	13	65	19	16	3	0	0	6	87
Dec. 10, 1937	22	0	0	27	0	5	64	9	9	0	0	0	0	41
Jan. 10, 1938	11	0	0	36	0	9	82	9	9	0	0	0	0	82
Jan. 21, 1938	11	0	0	9	0	0	91	9	18	0	0	0	9	91
Mar. 22, 1938	14	0	0	43	0	0	86	0	0	0	0	0	7	93
Total summer^{d}	85	0	0	25	0	40	12	47	26	3	1	0	9	82
Total fall, winter, and spring^{d}	148	2	0	32	1	5	67	28	9	1	1	1	9	70
Lumber and logs:														
Total summer	157	1	1	28	0	34	13	44	29	1	1	0	8	87
Total fall, winter, and spring	239	3	0	43	1	8	63	37	10	3	1	+	7	69

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a, b, c, and d See footnotes to table 1.

TABLE 3.—*Frequency of isolation of fungi from stained pine lumber and logs in northern Louisiana (Clarks), northern Mississippi (Bruce), and eastern Georgia (Savannah) on different dates*

Date and locality	Samples cultured	Percent of samples yielding—																			
			Ceratostomella										*Diplodia*								
		Alternaria	*exigua*	*ips*	*multiannulata*	*obscura*	*pilifera*	*pini*	*plurianulata*	spp.[a]	*Cadophora*	*Cladosporium*	*natalensis*	sp.	*Endoconidiophora coerulescens*	*Graphium*	*Helminthosporium*	*Leptographium*	*Pullularia*	Dark undetermined[b]	Nonstaining[c]
Northern Louisiana:																					
Apr. 6, 1937	12[e]	8	0	0	0	0	42	8	0	8	0	0	8	0	0	8	0	8	0	25	67
Apr. 19, 1937	18[f]	28	0	6	0	6	0	28	6	22	0	0	22	0	0	0	0	0	6	17	72
July 17, 1937	9[e]	0	11	56	0	0	0	0	0	0	11	0	0	22	0	0	0	0	0	0	67
Dec. 30, 1937	3[e]	0	0	0	0	0	0	0	0	0	0	100	0	0	0	0	0	0	0	0	
June 15, 1938	17[f]	12	0	29	0	0	47	0	0	0	0	0	0	35	0	0	0	0	0	0	47
Aug. 3, 1938	16[e]	0	0	25	0	0	31	0	0	0	0	0	0	0	0	6	13	0	0	25	100
Aug. 23, 1938	12[e]	0	0	50	0	0	8	0	0	0	0	0	25	8	8	0	0	0	0	8	92
Sept. 7, 1938	10[e]	0	0	20	0	0	20	0	0	0	0	0	10	50	0	0	0	0	0	30	90
Total summer[d]	50	16	0	12	0	2	26	12	2	10	2	0	10	12	0	2	0	2	2	12	58
Total fall, winter, and spring[d]	47	0	2	36	0	0	17	0	0	0	0	6	9	17	2	2	4	0	0	17	89
Northern Mississippi:																					
Feb. 11, 1938	7[g]	14	0	0	0	0	71	0	0	0	0	0	0	0	0	0	0	0	14	14	100
Mar. 10, 1938	11[e]	64	0	9	0	0	27	0	9	0	0	0	0	36	0	0	0	0	0	0	91
Apr. 1, 1938	30[f]	23	0	3	0	0	63	0	3	3	0	0	0	0	0	7	0	0	0	10	100
Aug. 4, 1938	2[e]	0	0	0	0	0	100	0	0	0	0	0	50	0	0	0	0	0	0	0	100
Aug. 29, 1938	4[e]	50	0	0	0	0	100	0	0	0	0	0	50	50	0	0	0	0	0	25	100
Total summer	6	33	0	0	0	0	100	0	0	0	0	0	50	33	0	0	0	0	0	17	100
Total fall, winter, and spring	48	31	0	4	0	0	56	0	4	2	0	0	0	8	0	4	0	0	2	8	98
Eastern Georgia:																					
Feb. 28, 1938[h]	6[f]	0	0	0	67	0	83	0	0	0	0	0	0	0	0	0	0	0	0	0	100
July 11, 1938	10[g]	10	0	20	0	0	0	0	0	50	0	0	30	0	0	0	0	0	10	0	100

[a] [b] [c], and [d] See footnotes to table 1.

[e] Log stain samples only.

[f] Lumber-stain samples only.

[g] Lumber- and log stain samples combined

[h] Fungi determined by fruiting on surface of sample, as excessive mold precluded culturing

From the data presented here and from other isolation work, it seems that the same staining fungi are important in Lousiana, Mississippi, and Alabama, and, with the possible exception of *Diplodia* sp., in northern Florida and in Georgia also.

STAINING FUNGI ISOLATED DURING DIFFERENT SEASONS

The isolations made from stained wood on different dates are listed in tables 1 to 4 and graphically shown in figure 2 for the important staining fungi isolated in southeastern Louisiana.

TABLE 4.—*Frequency of isolation of fungi from stained hardwood lumber and logs in northern Louisiana (Clarks), northern Mississippi (Bruce), and eastern Georgia (Savannah) on different dates*

Date and locality	Samples cultured	Per cent of samples yielding—											
				Ceratostomella			*Endoconidiophora*						
		Alternaria	*Cadophora*	*pluriannulata*	spp.[a]	*Diplodia natalensis*	*coerulescens*	*moniliformis*	*Graphium*	*Helminthosporium*	*Torula*	Dark undetermined[b]	Nonstaining[c]
Northern Louisiana:													
Apr. 6, 1937	13[e]	8	0	39	0	0	68	0	8	0	8	8	69
July 7, 1937	16[e]	0	0	19	0	75	6	6	31	0	0	25	37
Dec. 30, 1937	15[e]	0	0	73	0	0	60	0	13	0	0	7	100
July 1, 1938	15[f]	0	7	67	7	27	13	7	27	0	0	0	93
Aug. 3, 1938	10[g]	0	0	60	0	10	20	60	70	0	0	0	100
Sept. 7, 1938	11[e]	0	0	36	0	45	45	9	36	0	0	27	100
Total summer[d]	52	0	2	44	2	42	19	17	38	0	0	13	79
Total fall, winter, and spring[d]	28	4	0	57	0	0	64	0	11	0	4	6	86
Northern Mississippi:													
Apr. 1, 1938	7[e]	29	0	14	0	14	0	0	0	14	0	52	100
Aug. 4, 1938	12[e]	0	0	17	0	17	33	58	42	0	0	0	33
Eastern Georgia:													
Feb. 28, 1938	12[f]	0	0	25	0	0	75	0	0	0	0	8	67
July 11, 1938	29[f]	3	0	28	0	21	59	18	35	3	0	7	79

[a, b, c, and d] See footnotes to table 1.

[e] Log-stain samples only.

[f] Log- and lumber-stain samples.

[g] Lumber-stain samples only.

Fungi Isolated in Southeastern Louisiana

The outstanding seasonal changes in the staining flora isolated from pines are: (1) the relatively great abundance of *Diplodia natalensis* during the period from late June to the middle of November, and (2) the increase in the numbers of *Ceratostomella pilifera* after the numbers of *D. natalensis* decreased in the fall. In logs, *D. natalensis* did not show so long a period of high frequency as in lumber, and the peak of its frequency did not come until later, early October. However, it should be remembered that the isolations from logs were made at unknown lengths of time after infection, while those from lumber were made promptly after infection.

Diplodia sp. showed no marked seasonal changes. The numbers of *Ceratostomella ips* probably paralleled the changes in the *Ips* beetle population. In the yard, *C. ips* dropped from a June high, the month when beetles were most prevalent in the seasoning piles, while, in logs, *C. ips* stayed relatively high during the winter.

From the data it is apparent that the numbers of isolations of *Endoconidiophora coerulescens* varied inversely with those of *Diplodia natalensis. E. coerulescens* was abundant except for the period from late June to the middle of October when *D. natalensis* was of high frequency. During this period *E. coerulescens* was isolated in sufficient numbers to be really important only on June 21 and October 6 from lumber and June 29 and September 3 from logs. From November to May, *D. natalensis* was isolated infrequently, while *E. coerulescens* was by far the most prevalent of the major staining fungi in hardwood lumber and logs.

Ceratostomella pluriannulata and *Graphium rigidum* showed no marked seasonal changes in abundance, being isolated in large numbers at times during both the hot and the cool seasons.

Fungi Isolated in Northern Louisiana, in Northern Mississippi, and in Eastern Georgia

From pines, *Ceratostomella pilifera* was isolated more frequently in northern Louisiana, northern Mississippi, and eastern Georgia during the summer of 1938 than it was in northern and southeastern Louisiana during the summer of 1937; in fact, on several occasions it was the predominant fungus isolated from stained pines during June, July, and August, 1938. *Diplodia natalensis* never appeared as the predominant fungus on pine in northern Louisiana and northern Mississippi, although it assumed an important place among the isolates secured during the summer.

Isolations made from stained hardwoods in northern Louisiana during 1937 showed the same seasonal distribution of *Diplodia natalensis* and *Endoconidiophora coerulescens* as did those from southeastern Louisiana during the same period. However, during the summer of 1938 the numbers of isolations of *E. coerulescens* remained somewhat higher in Louisiana and Mississippi, and the numbers of *D. natalensis* somewhat lower than in Louisiana during the previous summer, although the same general tendencies were evident. At Savannah, Georgia, there was little evidence in the data of a summer drop in the incidence of *E. coerulescens.*

FACTORS INFLUENCING SEASONAL PREVALENCE

One of the factors thought to be important in determining seasonal changes in frequency of staining fungi is temperature, influencing the growth rate and fruiting of the fungi concerned. It was thought that high temperature during the summer might at least reduce the importance of some staining fungi by lowering their growth rate and also might lower their frequency by reducing the amount of inoculum produced, if not by killing them outright, under certain conditions.

Duplicate cultures of 14 isolates of 7 species were grown on 2½ per cent malt agar in constant-temperature incubators at 28, 30, 32, 34, and 35.5 degrees C. and the radial growths recorded (Table 5).

TABLE 5.—*Comparison of 72-hour radial growths, in mm., of species of Diplodia, Ceratostomella, Graphium, and Endoconidiophora on malt agar at different temperatures*

Species	Number of isolates	Degrees Centigrade				
		28	30	32	34	35.5
D. natalensis	4	57.6	56.4	52.6	44.2	32.9
C. ips	2	21.8	21.5	22.0	21.0	14.5
Diplodia sp.	2	20.8	18.8	14.5	5.0	2.0
C. pilifera	1	12.0	9.5	8.5	2.0	+
C. pluriannulata	1	10.0	8.0	6.5	+[a]	0.0
G. rigidum	2	7.5	6.2	6.2	1.5	0.0
E. coerulescens	2	22.8	13.5	8.5	0.0	0.0

[a] + indicates a trace of growth.

From these growth data it appears that summer temperatures would have little effect on the growth of *Diplodia natalensis* and *Ceratostomella ips,* might have a marked effect on *Endoconidiophora coerulescens,* and would influence to a less extent the other major stain fungi. *E. coerulescens,* failing to survive at 35.5° C. for 3 days, is the species most difficult to keep in culture without refrigeration.

The mean daily high air temperature and the number of days with daily maximum temperature of 90° F. (32.2° C.), or over, is shown in figure 3 for the summer months at the localities where isolations were made.

In any correlation of recorded air temperatures and incidences of staining fungi it must be remembered that temperatures inside fresh seasoning piles of lumber are lower than outside air temperatures during hot weather because of the cooling effect of evaporating moisture from the drying lumber. Lindgren[4] showed that in pine piles 1 to 40 days old the temperature was about 25° to 29° C., with outside temperature up to 34° C. A few data taken at Clarks in 1938 show that temperature inside fresh hardwood piles is likewise under 30° C., with outside temperature ranging up to 35° C. Thus there is little probability that during the first weeks of seasoning high temperature inside the piles is a limiting factor in the development of any of the major stain fungi. In some more openly stacked pine piles the temperature approaches that outside the piles, and even though high temperature in such piles greatly limits the development of such fungi as *Ceratostomella pilifera,* the factor of rapid surface drying probably is more important.

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[4] Lindgren, R. M. Unpublished thesis, University of Minnesota. 1937.

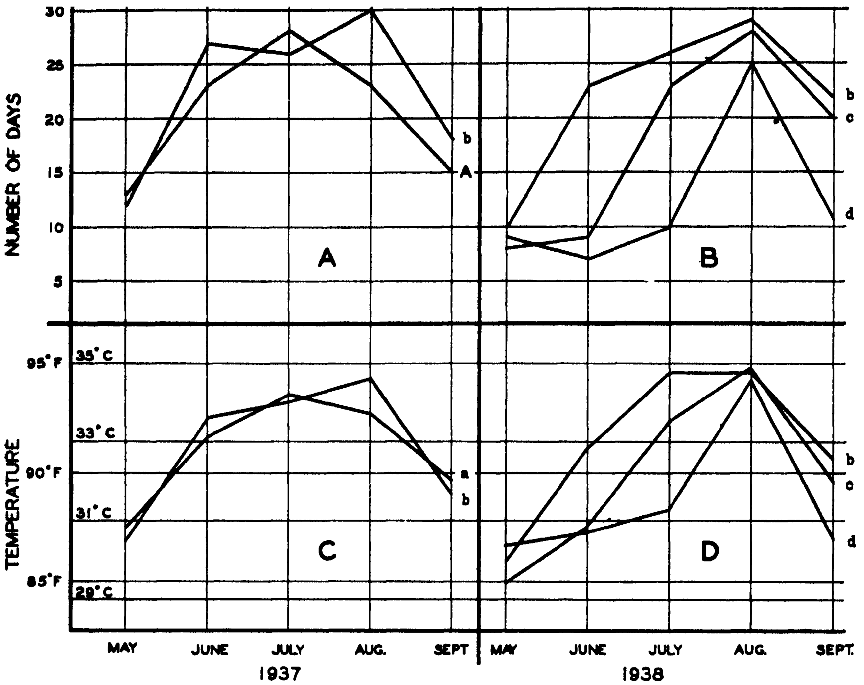

FIG. 3. A and B, number of days with maximum temperatures of 90° F. (32.2° C.) or above; and C and D, mean daily maximum temperatures, during the summer of 1937 and 1938, at the localities where isolations were made. Data taken from climatological data, monthly section reports, of the United States Weather Bureau. a, Southeastern Louisiana, Bogalusa; b, Northern Louisiana, Urania, near Clarks; c, Northern Mississippi, Tupelo, near Bruce; d, Eastern Georgia, Savannah.

It seems probable that the effect of high summer temperature on incidence of staining fungi is one of reducing growth and fruiting on exposed wood surfaces, both the high temperature as such and the concomitant rapid drying being involved. The commonest and most consistent places of fruiting of most of the hardwood-staining fungi in mill yards are log and lumber ends, where log-staining fungi usually can be found fruiting . In pine yards there is probably more fruiting on new foundations and flat surfaces of lumber. Log and lumber ends usually are exposed during part of the day to direct sun and apparently reach temperatures that kill *Endoconidiophora coerulescens* at the surface, reducing the amount of inoculum produced. Perithecia of *E. coerulescens* were found on a number of log-stained samples from which the fungus was not isolated. On a few days when *E. coerulescens* was not isolated from samples bearing perithecia, the temperature in the culture laboratory exceeded the maximum for its growth, but on others the laboratory temperature was well below the maximum, indicating killing in the field. There is no evidence that *Ceratostomella pilifera, C. pluriannulata,* and *Graphium rigidum* are killed by high temperatures in mill yards, but temperatures preventing fruiting on exposed surfaces are common. As *Diplodia natalensis, D.* sp., and *C. ips* grow at such high temperatures, it is improbable that short periods of high temperature affect their fruiting to a great extent. Of course, with hardwood lumber that is not chemically treated to prevent staining, or even with treated lumber under very severe seasoning conditions, any of the staining fungi can be expected to fruit during hot weather in the cooler, protected interiors of seasoning piles. Under such conditions, the populations of *Endoconidiophora coerulescens* may remain relatively high during hot weather. With the general practice of applying chemical solutions to freshly sawed hardwood lumber in the Southern States, little fruiting of staining fungi inside hardwood seasoning piles seems to occur.

The possible influence of high summer temperature in seasoning yards appears to be active, partly at least, in the woods and during the storage and transit of logs, because the seasonal trends of log-staining fungi are similar to those of lumber-staining fungi. However, the presence of perithecia of *Endoconidiophora coerulescens* on log-stain samples from which this fungus was not isolated suggests that more inoculum of *E. coerulescens* is produced in the woods than in the mill yards during the summer months and that the most important effect of high summer temperatures on log-staining fungi occurs during storage and transit of logs.

At Savannah, where there was no sharp rise in summer maximum temperatures (Fig. 3) until after the isolations were made in August, there was no indication of a drop in the incidence of any staining fungi during the summer. In 1938 the high maximum summer temperatures at Tupelo, Miss. (near Bruce), and Urania, La. (near Clarks), did not come until a month later than in 1937 at Bogalusa, La., and Urania, La. Correspondingly, the incidences of *Endoconidiophora coerulescens* and *Ceratostomella pilifera* did not drop so noticeably during the summer of 1938 at Bruce and Urania as in 1937 at the places studied.

The frequency of *Ceratostomella ips* is probably closely correlated with that of *Ips* beetles. In the Southern States, *Ips*[5] is generally active from March to December (7), and some active *Ips* were observed in pine seasoning piles during the warm days in January and February, 1938. During the cooler months *Ips* activity was apparently greater in the woods than in lumber yards, since the number of *C. ips* isolated from logs stayed relatively high during the winter. Although the close association of *C. ips* and *Ips* has been established (6), means of dissemination other than by *Ips* are indicated by the facts that *C. ips* was isolated from restricted stain areas around tunnels of an ambrosia beetle (*Xyleborus*), from the air, and from adults of *Orthotomicus caelatus* (Eich.) collected in mill yards. However, the present studies substantiate the previous work, indicating that the frequency of *C. ips* is dependent almost entirely on the frequency of *Ips* beetles.

The reason for the drop in the frequency of *Diplodia natalensis* during the cooler months is still largely a matter of conjecture. Secondary spores (conidia) are formed by all the major staining fungi, except the species of *Diplodia*, within a few hours of spore germination, and constitute an abundant source of inoculum. The *Diplodias* produce only pycnidiospores, which, in the laboratory, are formed only after 2 or more weeks. In mill yards very little fruiting of *Diplodia* was observed. However, during the summer, *D. natalensis* is a common fungus on a variety of crops such as cotton and sweet potato, and it seems possible that the inoculum of this fungus causing wood stain is produced largely on such plants. The drop in frequency of *D. natalensis* in the fall may be related in some way to a seasonal cessation of inoculum production on the variety of plants that are hosts to this species.

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[5] *Ips calligraphus* Germ., *Ips grandicollis* Eich., and *Ips avulsus* Eich.

SUMMARY

Isolations and artificial inoculations showed that the major stain damage in lumber and logs in the Southern States is caused by 7 fungi: *Endoconidiophora coerulescens, Ceratostomella pluriannulata, Diplodia natalensis,* and *Graphium rigidum* in hardwoods; and *Ceratostomella pilifera, C. ips, Diplodia natalensis,* and *Diplodia* sp. in pines. Besides these, a number of fungi, apparently of less importance, were isolated.

A *Diplodia,* apparently previously unreported as a staining fungus, and *D. natalensis* are described.

The same fungi were isolated from stained logs and lumber.

Apparently there are no differences in the species of staining fungi or in their frequency in different species of pines or in different species of hardwoods.

The only indication of geographical restriction of any of the major staining fungi within the Southern States was the absence of *Diplodia* sp. among the isolates secured in eastern Georgia.

D. natalensis is of prime importance only during the hot summer months, although it may occur to some extent during the cooler months; *C. pilifera* is important the year round, but is usually relatively more frequent during cooler months, when *D. natalensis* is of low frequency; *C. ips, Diplodia* sp., *C. pluriannulata,* and *Graphium* showed little seasonal fluctuation. *E. coerulescens* was by far the most prevalent of the major stain fungi on hardwoods during most of the year, although its incidence dropped very low during much of the hot summer-month period.

There is a general, although not absolute, correlation between the seasonal frequency of staining fungi and the temperature relations for their growth.

The influence of high temperature on fruiting is thought to be the chief factor in reducing the numbers of certain species during hot weather. *Ceratostomella ips* distribution probably is determined largely by the frequency of *Ips* beetles. The virtual disappearance of *Diplodia natalensis* during the cooler months may be due to seasonal cessation of production of inoculum on crop plants.

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EFFECT OF NITROGEN SUPPLY OF SWEET CORN ON THE WILT BACTERIUM

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The influence of soil fertility on plant diseases has been attributed usually to a direct effect of certain nutritional elements upon the plants. For example, it is often assumed that an increase in the nitrogen supply increases the severity of certain diseases by rendering the plant more succulent, hence more susceptible to invasion. Trelease and Trelease (14) have pointed out, however, that some factor, or factors, other than plant growth determined susceptibility of wheat to *Erysiphe graminis* DC. A similar conclusion was reached by Spencer and McNew (13), who found that the changes in severity of sweet-corn wilt induced by variations of the nitrogen, phosphorus, or potassium supply were not always correlated with the growth of the host. They suggested, among other things, that the nutritional elements might directly affect the invasiveness of the causal bacterium, *Phytomonas stewarti* (E.F.S.) Bergey *et al.* This possibility warrants consideration because the bacterium multiplies, during the early stages of invasion, almost exclusively in the tracheal tubes, which are known to conduct inorganic salt solutions. With this possibility in mind, studies were undertaken to determine what effect the nitrogen supplied to sweet-corn seedlings in sand cultures would have on the nitrogen content of the tracheal sap and on the growth rate and inherent virulence of the bacterium. The results of the studies are presented in this paper.

MATERIALS AND METHODS

Sweet-corn seedlings of the variety Golden Bantam were grown in sand culture, as previously described (13). Each 4-inch pot containing 3 seedlings was supplied with 100 cc. of nutrient solution 3 times a week. The basic nutrient solution used in all experiments contained 0.429 g. KH_2PO_4, 0.213 g. $CaCl_2 \cdot 2H_20$, and 0.875 g. $MgSO_4 \cdot 7H_2O$ per l. This solution was supplemented with traces of manganese, boron, and iron. Sufficient NH_4NO_3 was added to give the desired concentration of nitrogen (20 to 2000 p.p.m.).

Approximately 100 seedlings were placed on each nutrient treatment. Thirty of these were inoculated when 10 days old by injecting a broth culture of *Phytomonas stewarti* into their crowns. All of the cultures used, except B–102 and B–1311, have been previously described (7). Culture B–102 was a highly virulent strain isolated from naturally infected sweet corn in New Jersey in August, 1938, and culture B–1311 was a single-cell isolate from culture B–1011. The remaining non-inoculated seedlings were cut off about 2 in. above the sand surface and their tracheal exudate collected in 2-cc. Wassermann tubes. In case the seedlings had not been fed on the day of inoculation, the exudate was collected the following night after feeding. All deviations from these procedures are noted in the text.

The tracheal exudate from seedlings on each treatment was mixed and 3 aliquots of 2 cc. each were analyzed for total nitrogen (12). The remainder of the exudate was mixed with an equal volume of dextrose agar (2 and 3 per cent, respectively) and tested as a nutritive substrate for *Phytomonas stewarti.* Part of the exudate medium from each treatment was supplemented with 30 p.p.m. of nitrogen in the form of NH_4NO_3 or $(NH_4)_2SO_4$. The media were then adjusted to pH 7.0, placed in tubes, autoclaved at 20 lb. pressure for 15 min., and slanted. Duplicate tubes of each medium were seeded with inoculum from a 24- to 48-hr. culture on nutrient-dextrose agar. The density of growth was observed after 24 and 48 hours.

For comparative purposes, in some tests the exudate was sterilized by filtration through a Berkefeld "N" candle and added to autoclaved agar. Also in some tests the tops were frozen, ground up and extracted, and the extract added to plain agar. The media prepared by these various modifications gave results similar to those reported for media prepared by the standard procedure.

Infected seedlings receiving the different nutrient treatments were analyzed for variants of *Phytomonas stewarti* by the following procedure. A piece of infected tissue was placed in 85 per cent alcohol for ½ minute, rinsed in sterile distilled water, macerated in nutrient-dextrose broth, and incubated for about 1 hour to allow the bacterial cells to become separated from each other. The broth suspension was diluted and dispersed in melted nutrient(Difco)-dextrose agar in dilution plates. Distinctly separated colonies were isolated 48 hours later. A 24-hr. broth subculture of each isolate was tested for virulence by injection into 15 to 20 sweet-corn seedlings growing in soil. In some tests, the seedlings were grown in 4-inch pots, inoculated when 10 days old, and observed for wilting 10 days later. The virulence of the cultures in such tests is expressed by an infection index calculated as the average number of necrotic lesions produced per leaf (13). In other tests, where many cultures were used, the seedlings were grown in flats, inoculated when 7 days old, and observed for infection 14 days later. Virulence in such tests is expressed as the percentage of leaves invaded.

EXPERIMENTAL RESULTS

Tracheal Nitrogen Necessary for Growth of *Phytomonas stewarti*

Sweet-corn seedlings were grown in sand and supplied with nutrient solutions containing either 0, 20, 200, or 1000 p.p.m. of nitrogen. A comparable group of seedlings was grown in ordinary greenhouse compost as a control. Some of the seedlings were inoculated with culture B–102 and the remainder were used for sap analysis either on the day of inoculation or at the time records of infection were being taken 10 days later. Since the exudates collected on the two dates gave comparable results, only the data on exudates taken at the latter date are presented in table 1.

TABLE 1.—*The relation of the nitrogen content of tracheal exudate, from seedlings supplied different amounts of nitrogen, to the growth and invasiveness of Phytomonas stewarti*

Treatment of plant		Severity of wilting				Nitrogen content of exudate	Growth of bacteria[a] on exudate supplemented with	
Nitrogen supplied	Inoculation	No. test plants	No. of leaves	Percentage leaves Invaded	Percentage leaves Killed		H_2O	NH_4NO_3
p.p.m.						*p.p.m.*		
0	B-102	30	116	47.4	0.0			
0	None	29	116	0.0	0.0	21	tr, tr	+++, +++
20	B 102	30	128	51.6	1.6			
20	None	30	133	0.0	0.0	21	+, +	++, +++
200	B 102	27	136	74.3	15.4			
200	None	30	161	0.0	0.0	121	+++, +++	+++, +++
1000	B 102	30	140	83.7	20.0			
1000	None	30	162	0.0	0.0	492	+++, +++	+++, +++
Soil Ck.	B 102	30	144	59.7	2.1			
Soil Ck.	None	29	150	0.0	0.0	112	++, ++	+++, +++

[a] tr, possible growth in isolated spots along streak; +, faint growth; ++, definite growth along entire streak; +++, heavy growth along streak and accumulation of bacteria at base of slant.

The data obtained are typical of those from several such tests made at different seasons of the year. The seedlings deprived of nitrogen were not severely invaded. Their tracheal sap contained very little nitrogen and was a very poor nutrient substrate for *Phytomonas stewarti*. The bacteria rarely produced more than a faint trace of growth along the streak. This poor growth apparently resulted from the deficiency of nitrogen, since the exudate was converted into an excellent substrate by the addition of NH_4NO_3. The seedlings receiving 20 p.p.m. of nitrogen also had very little nitrogen in the tracheal sap. The bacteria produced a rather mediocre growth on this exudate. The tracheal exudates from seedlings supplied with 200 and 1000 p.p m. of nitrogen were relatively rich in nitrogen and served as an excellent culture medium for the bacteria. As shown in figure 1, the bacteria not only covered the slant but also accumulated at its base.

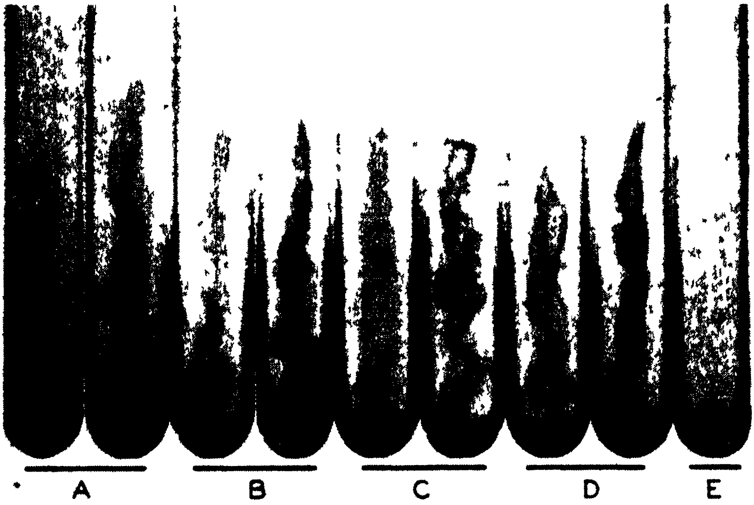

FIG. 1. Growth of *Phytomonas stewarti* after 48 hours on dextrose agar supplemented with water (E) and tracheal exudates from seedlings receiving 0 (A), 20 (B), 200 (C), and 1000 (D) p.p.m. of nitrogen. The second tube in each pair was supplemented with NH_4NO_3. (Photograph by J. A. Carlile.)

This test would indicate that the nitrogen supplied the seedlings is taken up into the tracheal tubes and that the amount present in the tracheal sap determines its suitability as a culture medium for *Phytomonas stewarti*. Well-nourished seedlings were more severely invaded than undernourished ones, because the bacteria apparently received sufficient nitrogen for rapid multiplication The seedlings grown in soil fit into this scheme perfectly, being intermediate in all respects to the seedlings grown in sand supplied with solutions containing 20 and 200 p p m. of nitrogen.

Further evidence of the dependence of the bacteria on the nitrogen supply in the tracheal sap was obtained by analyzing the exudate at different intervals after the seedlings had received nutrient solutions containing either 0 or 200 p.p m. of nitrogen. The seedlings deprived of nitrogen never contained more than a trace of nitrogen in their tracheal exudate, and the bacteria grew very poorly on this exudate unless it was supplemented with NH_4NO_3 (Table 2). Tracheal exudate from seedlings supplied with nitrogen was relatively rich in nitrogen immediately after feeding, and supported bacterial growth very well. After 31 hours, however, the tracheal exudate from comparable seedlings had only 15 per cent as much nitrogen. The bacteria grew less vigorously on this exudate with the reduced nitrogen content.

TABLE 2.—*Nitrogen content of tracheal exudate collected from seedlings at different intervals after supplying them with nitrogen-deficient and nitrogen-containing solutions*

Nitrogen supplied	Interval after feeding	Nitrogen content of exudate	Growth of bacteria on exudate supplemented with	
			H_2O	NH_4NO_3
p.p.m.	*hrs.*	*p.p.m.*		
0	7–23	7	tr, tr	++, ++
0	31–47	7	tr, tr	++, ++
200	7–23	270	+++, +++	+++, +++
200	31–47	41	++, ++	+++, +++

The foregoing data show that nitrogen is necessary for good bacterial growth in the tracheal sap. If nitrogen affects the host-parasite complex merely by influencing the growth rate of the bacteria, then seedlings dwarfed by nitrogen starvation should be invaded as soon as nitrogen is made available. This supposition was tested by comparing the severity of invasion in 4 groups of seedlings grown in sand and supplied with nutrient solutions as follows: Two groups were deprived of nitrogen until the day of inoculation when one was supplied with a nutrient solution containing 200 p.p.m. of nitrogen. The third and fourth groups received 200 and 1000 p.p.m. of nitrogen, respectively, from the beginning of nutrient treatment. Exudate was collected from the various groups immediately after inoculation and tested for nitrogen and ability to support bacterial growth. The data obtained (Table 3) show that seedlings supplied with nitrogen for the first time on the day of inoculation had about 20 per cent more nitrogen in their exudate than seedlings that had received this amount of nitrogen at regular intervals. The previously deficient seedlings were growing slowly and probably had not yet assimilated the absorbed nitrogen. They were, however, just as severely invaded as those that had received the same nitrogen treatment from the beginning. Both groups had about twice as many leaves invaded as seedlings deprived of nitrogen throughout the test. These differences in invasiveness of the bacterium were closely correlated with the ability of the exudate to support bacterial growth. The test shows that the severity of wilting was determined by the nitrogen content of the tracheal sap on the day of inoculation (and presumably thereafter) and not by the relative growth rate of the host. It should be pointed out that the seedlings previously deprived of nitrogen began to regain their normal green color and to resume growth about 6 days after being supplied with nitrogen.

Amount of Nitrogen Required for Growth of *Phytomonas stewarti* in Culture

Phytomonas stewarti grew well on agar media prepared with exudates containing 40 p.p.m. or more of nitrogen (Tables 1, 2, 3). Thus, it would seem that the minimum nitrogen requirement for the bacteria is about 20

TABLE 3.—*Severity of infection in seedlings supplied with differing amounts of nitrogen before and after inoculation with Phytomonas stewarti*

Nitrogen supplied		Severity of wilting[a]				Nitrogen content of exudate	Growth of bacteria on exudate supplemented with		
Before inoculation	After inoculation	No. test plants	No. leaves	Percentage leaves					
				Invaded	Killed		H_2O	NH_4NO_3	$(NH_4)_2SO_4$
p.p.m.	*p.p.m.*					*p.p.m.*			
0	0	30	160	26.2	0.0	33	+, +	++, ++	+++, +++
0	200	30	202	53.5	8.9	328	++, +++	+++, +++	+++, +++
200	200	30	232	52.2	7.8	270	+++, +++	+++, +++	+++, +++
1000	1000	30	218	58.3	9.6	616	+++, +++	+++, +++	+++, +++

[a] Observed 16 days after inoculation.

p.p.m., since the exudate had been diluted with melted agar. This figure cannot be accepted unconditionally because the micro-Kjeldahl method used was not accurate for determining such small amounts. For example, in final titrations with 0.02 N HCl, an error of one-half drop would change the nitrogen value by 7×10^{-6} g. It was necessary, therefore, to check the observations on tracheal exudates by studying the bacterial growth on media of known composition.

A synthetic agar medium consisting of the nitrogen-deficient solution, dextrose (1 per cent) and agar (1.5 per cent), was adjusted to pH 7.0 and divided into 9 aliquots. The different aliquots were supplemented with sufficient NH_4NO_3 to give a range of 0 to 280 p.p.m. of nitrogen, then tubed and autoclaved. Duplicate tubes of each medium were seeded with the highly virulent strains B–102, B–1011, B–1311, and B–91, and an almost avirulent strain B–1211. All strains grew poorly, if at all, on media containing 14 p.p.m. or less of nitrogen (Table 4). However, all virulent strains grew well on media containing 28 p.p.m. or more of nitrogen. The failure of the weakly virulent culture to grow on these media was to be expected, since it was known (7) to be unable to use inorganic nitrogen.

TABLE 4.—*Growth of virulent and avirulent strains of Phytomonas stewarti on synthetic agar medium containing different amounts of nitrogen (as NH_4NO_3)*

Nitrogen content of medium	Growth of *Phytomonas stewarti* strains				
	Virulent				Avirulent
	B 102	B-1011	B-1311	B 91	B-1211
p.p.m.					
0.0	– –, tr	tr, tr	tr, tr	tr, tr	tr, tr
2.8	tr, +	+, +	+, +	+, +	tr, tr
5.6	+, +	+, +	+, +	+, +	– –, tr
14.0	+, ++	++, ++	++, ++	++, ++	tr, tr
28.0	++, +++	+++, +++	++, +++	+++, +++	– –, tr
56.0	+++, +++	+++, +++	++, ++	+++, +++	tr, +
84.0	+++, +++	+++, +++	+++, +++	+++, +++	tr, +
140.0	+++, +++	+++, +++	+++, +++	+++, +++	tr, tr
280.0	+++, +++	+++, +++	+++, +++	+++, +++	– –, tr

The virulent strains required about the same amount of nitrogen for growth in this synthetic medium as in tracheal-exudate media. Even though this minimum nitrogen requirement appears surprisingly low, an even lower figure might be obtained by using $(NH_4)_2SO_4$ as the nitrogen source. Most strains of *Phytomonas stewarti* use nitrates with difficulty, if at all (7).

Virulence of Bacteria from Plants Receiving Different Amounts of Nitrogen

In addition to controlling the growth rate of *Phytomonas stewarti*, the nitrogen supply of sweet-corn seedlings might also directly affect the inherent virulence of the bacteria. Cultures of the bacteria were, therefore, tested for virulence both before and after passage in seedlings supplied with different amounts of nitrogen. Several single-colony isolates from each culture were tested, because it has been shown that mixtures of variants exist both in culture and in the host (6, 7). Culture B–1011 was transferred to nutrient-dextrose broth about 5 months after its isolation (6). After 24 hours' incubation, the broth culture was thoroughly agitated and a portion of it analyzed for variants that differed in virulence. The remainder of the culture was injected into 10-day sweet-corn seedlings receiving either 0 or 540 p.p.m. of nitrogen. After 57 days in the plants, bacteria were reisolated and tested for virulence. Sixty single-colony isolates from the original broth culture and 40 isolates from a plant on each of the nutrient treatments were tested and classified for virulence according to their infection indexes. The data obtained are presented in figure 2.

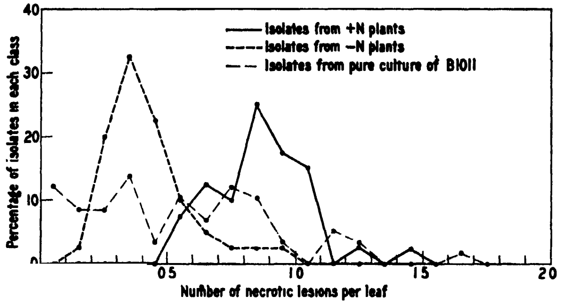

FIG. 2. Virulence of single-colony isolates obtained from a virulent culture of *Phytomonas stewarti* at the time of inoculation and after incubation in sweet-corn plants receiving 0 and 540 p.p.m. of nitrogen for 57 days.

The original subculture of B–1011 contained variants that ranged from almost avirulent to highly virulent. The portion of the culture incubated in a nitrogen-fed plant lost all of the slightly virulent strains and retained for the most part only strains of average virulence. On the other hand, the portion of the culture incubated in the nitrogen-deficient plant consisted almost entirely of weakly virulent strains. Only 3 isolates of the highly virulent type were recovered from this plant. The predominating strains in the 2 plants were very different since those from the nitrogen-deficient plant caused small lesions but no wilting, whereas those from the nitrogen-fed plant caused dwarfing, wilting, and sometimes death (Fig. 3). Comparable differences were observed between isolates from duplicate plants on the 2 treatments.

The 2 types of isolates obtained from these plants were typical of the species in regard to cultural characteristics and physiological properties. These properties have been described elsewhere (7) for cultures numbered B–211 (nitrogen-deficient) and B–311 (nitrogen-fed). The cultures differed only in their colony type. The weakly virulent isolates from the nitrogen-deficient plants produced a rather dry, firm, filiform streak on agar, while the other isolates produced a spreading liquid type of growth. Colonies of the weakly virulent strain were semi-cratered and firm on nutri-

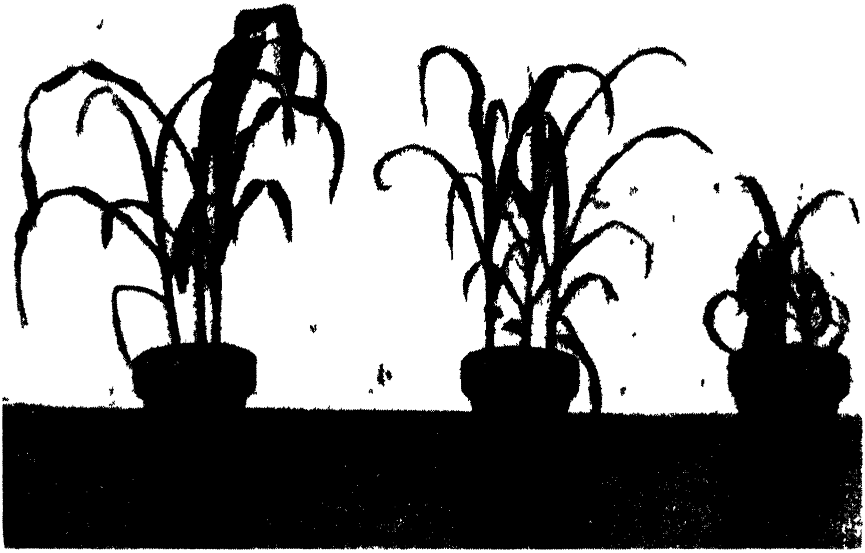

FIG. 3. Sweet corn seedlings inoculated with (from left to right) tap water, a typical weakly virulent isolate from a nitrogen deficient plant, and a typical highly virulent isolate from a nitrogen-fed plant. The seedlings were inoculated when 10 days old and photographed 12 days later. (Photograph by J. A. Carlile.)

ent-dextrose agar and were similar to the variant types described by McCulloch (5), Wellhausen (16), and Ivanoff *et al.* (4). One of these cultures was retained for observation over a 2-year period. It reverted to the smoother, spreading type during this period but did not change in virulence.

This experiment with culture B–1011 was repeated about a year later with comparable results (Fig. 4). However, the weakly virulent strains obtained from nitrogen-deficient plants did not differ from the more virulent ones in regard to their cultural characteristics.

In the repetition with culture B–1011, additional isolations were made on the 14th and 36th day after inoculation. On the 14th day, nitrogen-deficient plants yielded 4 isolates with an average index of 0.56 ± 0.07, and on the 36th day 17 isolates with 0.57 ± 0.13 and 7 with 0.61 ± 0.08. The nitrogen-fed plants, on the other hand, after 14 days yielded 7 isolates with an average index of 0.56 ± 0 08, 6 with 0.67 ± 0.16, and 8 with 0.84 ± 0.13, and after 36 days 13 isolates with 1.02 ± 0.17 and 14 with 1.00 ± 0.13. These data suggest that the strains in the nitrogen-fed plants were replaced by more virulent ones between the 14th and 36th days, while there was no change in the strains in the nitrogen-deficient plants. It should be remembered, however, that these apparent changes were determined by comparing isolates from only 2 or 3 plants on the 2 treatments at each date and might be due to errors in sampling.

On the 36th day, one plant of the nitrogen-fed series was observed to have a small, yellow lesion as the only symptom. Since most nitrogen-fed

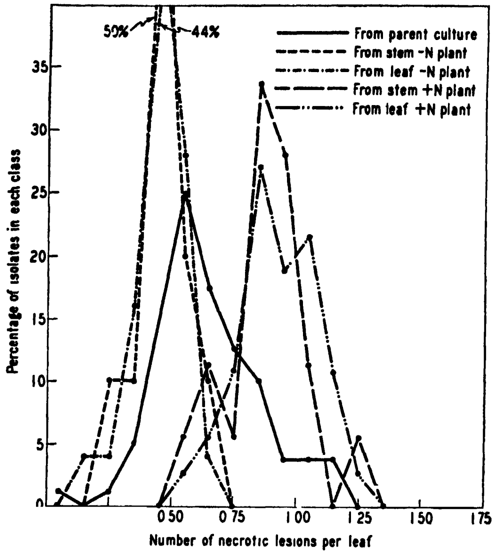

FIG. 4. Virulence of single-colony isolates obtained from a culture of *Phytomonas stewarti* at the time of inoculation and after incubation in sweet-corn plants receiving 0 and 400 p.p.m. of nitrogen for 36 days. Eighty single-colony isolates from the parent culture, 10 from the stem and 25 from the leaf of a nitrogen-deficient plant, and 18 from the stem and 37 from the leaf of a nitrogen-fed plant were tested.

plants were severely invaded and killed, it was considered of interest to test the virulence of the bacteria in the yellow lesion. Seven single-colony isolates from the lesion had an average infection index of 0.35 ± 0.12. Although it is impossible to account for the low degree of virulence of these isolates, it might be postulated that the plant was, by chance, invaded only by weakly virulent strains at the time of inoculation. Since the bacteria were not invasive, they did not spread to the remainder of the plant. Inasmuch as the bacteria did not multiply extensively, they had no opportunity to produce highly virulent strains. These data show that bacteria may survive in plants receiving nitrogen without necessarily attaining a highly virulent state.

Since the preceding experiment indicated that the bacteria usually became more virulent in nitrogen-fed plants, an attempt was made to determine whether recently isolated, highly virulent cultures would, on the other hand, lose virulence during passage in nitrogen-deficient plants. Seedlings of a susceptible Golden Bantam top cross were grown in soil and in sand supplied with nutrient solutions containing 0 or 200 p.p.m. of nitrogen. The 3 groups of seedlings were inoculated with the highly virulent culture B–102 shortly after it had been isolated from naturally infected sweet corn and purified twice by single-colony isolation. Thirty days after inoculation, bacteria were recovered from 3 plants on each treatment and 30 isolates from each plant were tested for virulence. All of the 180 isolates from the plants supplied with nitrogen or grown in soil were highly virulent, invading 70 to 100 per cent of the leaves. Most of the isolates from the nitrogen-deficient plants were equally virulent, but a weakly virulent isolate (20–30 per cent invasion) was identified in 2 of the 3 groups. This test showed that the highly virulent strains may persist in nitrogen-deficient plants. However, the weakly virulent strains present in 2 of the 3 plants might have eventually replaced the more virulent strains.

In the foregoing tests on the effect of host passage, the bacteria were incubated for long periods in a single plant on each treatment. The nitrogen-deficient plants were practically dormant during this period, and the bacteria were more or less confined to the tissues invaded within a week or two after inoculation. The nitrogen-fed plants, on the other hand, grew rapidly and the new tissue was invaded by bacteria. Under such conditions the bacteria in the nitrogen-deficient plants did not have so good a chance to multiply as those in plants receiving nitrogen. In order to reduce this difference in experimental conditions as much as possible, a series of tests was made in which the bacteria were passed serially every 10th day from diseased to healthy seedlings on the respective nutrient treatments.

The seedlings were grown in sand cultures and supplied with nutrient solutions containing either 0, 100, or 2000 p.p.m. of nitrogen. Four groups on each treatment were inoculated with culture B–1311. Ten days after inoculation, diseased tissue from each group was macerated in a sterile mortar, suspended in nutrient-dextrose broth, and the coarser plant parts were removed by filtration through sterile cheesecloth. The filtrate was then injected into healthy 10-day-old seedlings receiving the same nutrient treatment. After 11 host passages, the bacteria were recovered either from the leaf or stem of a typical seedling in each of the quadruplicate groups on each treatment. Several single-colony isolates from each group were tested for virulence. In general, the isolates obtained from the seedlings receiving 2000 p.p.m. of nitrogen were more virulent (Table 5) than those from seed-

TABLE 5.—*Average virulence of single-colony isolates of Phytomonas stewarti obtained from seedlings supplied with different amounts of nitrogen*

Source of isolates		No. of isolates tested	Percentage of leaves invaded
Plants supplied nitrogen	Plant part		
p.p.m.			
0	Leaf	10	31.6 ± 17.3[a]
0	Leaf	10	26.6 ± 24.8
0	Stem	3	21.7 ± 1.5
0	Stem	20	65.3 ± 9.9
100	Leaf	14	41.7 ± 8.0
100	Leaf	26	41.4 ± 11.1
100	Stem	27	75.3 ± 9.0
100	Stem	23	38.4 ± 9.8
2000	Leaf	10	59.6 ± 25.5
2000	Leaf	9	84.7 ± 13.4
2000	Stem	20	74.8 ± 13.3
2000	Stem	10	85.6 ± 6.3

[a] Standard deviation of mean.

lings receiving a low concentration of nitrogen or deprived of it. There were several irregularities observed, however, between the quadruplicate tests on each treatment. The variations observed in these different groups may indicate that the changes in variant populations came about rather slowly and that further host passages would have been necessary to complete the transformation of the cultures.

A Motile Contaminant Isolated from Sweet Corn

It was thought that some of the experimental variation reported in the previous experiment might be due to differences in resistance of plants of the open pollinated or top-cross Golden Bantam lines that were used, since Wellhausen (16) found that more virulent strains of *Phytomonas stewarti* developed in a resistant line of maize than in a susceptible line. The preceding experiment on host passage was, therefore, repeated using a susceptible inbred line of the Evergreen type. The results obtained with bacteria isolated after 5 and 8 passages confirmed those reported above. The bacteria isolated from the nitrogen-fed seedlings were usually more virulent than those from the nitrogen-deficient seedlings, but considerable variation occurred in the rate at which the change came about.

In the 7th and 8th passages, it was noted that 2 of the groups of nitrogen-deficient seedlings had small, irregular lesions. The bacteria isolated from these lesions produced light yellowish cream-color streaks on nutrient-dextrose agar slants that resembled those of *Phytomonas stewarti.* When inoculated into soil-grown seedlings, these cultures invaded 30 to 50 per cent of the leaves and caused pale yellow to necrotic leaf lesions similar to those produced by weakly virulent strains of *P. stewarti.* However, further study showed that the cultures were distinctly different from *P. stewarti* in that the bacteria were motile in hanging drops and produced a brown pigment on agar slants after 2 or 3 weeks. Because of these differences and the fact that the motile bacterium failed to produce typical strains of *P. stewarti* when passed serially in nitrogen-deficient and nitrogen-fed sweet-corn seedlings for 35 days, it is believed to belong in a different species. It is probably a soil- or air-borne contaminant that was accidentally injected during the serial host passages. Apparently it was sufficiently well adapted to the environment of the tracheal tubes to multiply. In this respect it is similar to other species of bacteria studied by Wellhausen (17). Considerable interest is attached to this motile species, which resembles *P. stewarti,* because Smith (10) originally described the corn-wilt bacterium as a motile organism. It was not until several years later that McCulloch (5) showed *P. stewarti* to be non-motile.

It was believed that the motile bacterium might be able to compete successfully with *Phytomonas stewarti* only in seedlings deprived of nitrogen, since it was not observed in nitrogen-fed seedlings. In order to test this possibility, the relative virulence of 2 isolates of each species was determined for seedlings receiving either 0 or 1000 p.p.m. of nitrogen. In

TABLE 6.—*Relative virulence of Phytomonas stewarti and the motile bacterium for seedlings supplied with different amounts of nitrogen*

Culture tested			Test plants		Condition of leaves				Infection index	Dry wt. per plant
No.	From plant	Characteristics	Nitrogen supplied	No. inoculated	Total No.	No. killed	No. of lesions	No. invaded		
			p.p.m.							*g.*
480	−N	Motile	0	12	61	0	19	16	0.31	0.20
470	−N	Motile	0	12	52	0	29	18	0.56	0.16
115	+N	Typical	0	11	47	0	25	20	0.53	0.18
116	+N	Typical	0	12	48	0	18	16	0.38	0.16
Control			0	15	79	0	0	0	0.00	0.23
480	−N	Motile	1000	12	69	3	28	21	0.54	0.55
470	−N	Motile	1000	12	69	4	25	18	0.54	0.30
115	+N	Typical	1000	12	48	42	12	6	2.88	0.14
116	+N	Typical	1000	12	54	40	19	10	2.57	0.24
Control			1000	14	93	0	0	0	0.00	0.60

seedlings deprived of nitrogen, the motile organism was just as invasive and caused as much reduction in dry weight as *P. stewarti* (Table 6). In seedlings receiving nitrogen, however, it was distinctly less invasive than *P. stewarti* and not much more virulent than in nitrogen-deficient seedlings.

Tests similar to those reported for the motile bacterium were conducted with strains of *Phytomonas stewarti.* Two of the weakly virulent isolates obtained from nitrogen-deficient seedlings and 2 highly virulent ones from nitrogen-fed seedlings were tested for virulence on seedlings receiving either 0, 200, or 1000 p.p.m. of nitrogen. The weakly virulent isolates were found to be practically as invasive as the highly virulent ones in nitrogen-deficient seedlings (Table 7), but distinctly less virulent in seedlings receiving moderate or heavy applications of nitrogen. The weakly virulent strains apparently were incapable of responding to the increased nitrogen supply which stimulated the virulent strains to become more aggressive.

TABLE 7.—*Virulence of single-colony isolates of Phytomonas stewarti for seedlings supplied with different amounts of nitrogen*

Isolate tested		Test plants		Condition of leaves				Infection index	Dry wt. per plant
No.	From plant	Nitrogen supplied	No. inoculated	Total No.	No. killed	No. of lesions	No. invaded		
		p.p.m.							*g.*
73	−N	0	28	125	0	22	18	0.18	0.30
82	−N	0	30	135	0	22	18	0.16	0.29
224	+N	0	29	128	0	55	40	0.43	0.31
225	+N	0	29	120	0	62	44	0.52	0.23
Controls		0	30	129	0	0	0	0.00	0.27
73	−N	200	29	139	0	18	17	0.13	0.74
82	−N	200	30	147	0	37	29	0.25	0.77
224	+N	200	30	140	6	118	66	0.97	0.51
225	+N	200	30	146	4	103	61	0.79	0.51
Controls		200	29	143	0	0	0	0.00	0.90
73	−N	1000	27	135	0	51	35	0.38	0.54
82	−N	1000	30	133	0	49	38	0.39	0.36
224	+N	1000	30	125	27	118	62	1.59	0.22
225	+N	1000	30	119	35	102	55	1.74	0.17
Controls		1000	29	138	0	0	0	0.00	0.47

DISCUSSION

Bacterial diseases are generally known to be most severe on rapidly growing succulent plants, particularly those receiving nitrogenous fertilizers. This relationship has been so obvious that one of the standard recommendations for the prevention of bacterial diseases, such as fire-blight of pears, caused by *Erwinia amylovora* (Burr.) Winslow *et al.,* is to avoid all cultural conditions that will stimulate excessive vegetative growth. Because of this close correlation between growth and disease, it has been generally assumed that nitrogen increases the severity of bacterial invasion merely by increasing the growth rate of the host. Although there is very little direct evidence to support this hypothesis, it is not improbable that succulent tissues of rapidly growing plants are more easily penetrated and disintegrated than those consisting of heavy-walled cells.

On the other hand, there is no good reason for assuming that the parasite is not likewise directly affected by the minerals absorbed by the host. The data presented in this paper show that the amount of nitrogen in the tracheal sap of maize directly affects the growth of *Phytomonas stewarti.* This direct effect is probably attributable to the fact that the bacterium lives almost exclusively in the tracheal tubes during the early stages of its invasion (3) and, therefore, depends upon the materials in the transpiration stream for its sustenance. It has been shown elsewhere (7) that virulent strains of this bacterium always use inorganic nitrogen and that the ability to use such nitrogen is closely associated with virulence of the parasite. The fact that strains that lose the ability to assimilate inorganic nitrogen also lose virulence suggests that there is little or no organic nitrogen in the tracheal sap available for bacterial growth. The present studies complete the evidence that this bacterium depends on inorganic nitrogen of the tracheal sap for its parasitic existence.

The data presented offer an explanation of why the severity of invasion is directly correlated with the total amount of nitrogen supplied the seedling rather than with the growth of the host resulting from such applications. The bacterium grew in tracheal sap that contained 20 to 40 p.p.m. of nitrogen, but did better on sap with about 200 p.p.m. These amounts were obtained in plants receiving nutrient solutions containing 200 p.p.m. of nitrogen, a concentration that was about optimum for host growth. Evidence was obtained that nitrogen was absorbed within about 8 hours (Table 3) and most of it assimilated within about 31 hours (Table 2). Thus, the bacterium had an adequate supply of nitrogen only about half the time, since the seedlings were fed every 2 days. The bacteria in seedlings receiving an excess of nitrogen (1000 p.p.m.) may have been more invasive because the seedlings were unable to use all of the nitrogen and thereby deprive the bacteria during the interval between nutrient applications.

The difference in virulence of bacteria in nitrogen-deficient and nitrogen-fed seedlings is not considered to be a major factor in explaining why nitrogen increases the severity of bacterial wilt. The changes in variant population came about so slowly and were usually so incomplete that they would not materially affect the host-parasite relationship during the early stages of invasion. The virulent strains were much more invasive than the weakly virulent ones when they had adequate nitrogen (Table 7). However, when they were rendered impotent by nitrogen starvation, they probably could not compete so successfully with the less virulent strains, and it became largely a matter of chance as to which would predominate in the host. In other words, the less virulent strains may have had any one of several physiological deficiencies that handicapped their invasiveness and placed them at a disadvantage in competing with the virulent strains, unless the latter were deprived of an essential nutrient such as nitrogen.

The tendency for attenuated strains to replace the virulent ones in seedlings rendered insusceptible by nitrogen starvation appears contrary to Wellhausen's (16) observation that virulent strains develop more readily in resistant inbred lines of maize than in susceptible ones. However, there is no direct conflict between the 2 observations because entirely different mechanisms probably are involved in the selection of strains. In nitrogen-deficient seedlings the virulent strains were handicapped by nitrogen starvation, whereas they very likely had all the nitrogen they needed in Wellhausen's resistant lines.

These observations on the effect of nitrogen on the host-parasite complex present only one aspect of the problem of plant nutrition in relation to disease. Such a direct effect on the parasite may not apply to other diseases in which the parasite is established in tissues other than the tracheal tubes. However, Nightingale (8) has offered evidence that the invasiveness of *Erwinia amylovora* in cortical tissues of apple twigs is correlated with the amount of organic nitrogen present, which in turn can be controlled by the amount of inorganic nitrogen supplied the tree. Furthermore, nitrogen is only one of the several nutritional elements that affect diseases of plants (9, 15, 2, 1, 11). For example, phosphorus and potassium affect the severity of sweet-corn wilt (13). Preliminary tests conducted during the present studies failed to show that these elements had any direct effect upon either the growth rate or virulence of the bacterium. The 2 elements may alter the host reaction.

SUMMARY

The addition of nitrogen to sweet-corn seedlings increased the severity of wilting caused by *Phytomonas stewarti.*

The amount of nitrogen supplied the seedlings determined the concentration of nitrogen in the tracheal sap and the suitability of this sap as a nutrient substrate for the pathogen. The bacteria were invasive in seedlings that contained as much as 20 to 40 p.p.m. of nitrogen in their tracheal sap. However, seedlings containing as much as 270 p.p.m. in their sap quickly assimilated the nitrogen between feeding periods, so that soon after feeding there was an insufficient supply for good bacterial growth.

There was a stronger tendency for virulent strains of the bacterium to develop in nitrogen-fed plants than in nitrogen-deficient ones. In some tests, however, virulent strains persisted in nitrogen-deficient plants for long periods. Tests showed that weakly and highly virulent strains were about equally aggressive in nitrogen-deficient seedlings but that highly virulent strains were much more invasive in seedlings receiving liberal amounts of nitrogen.

A motile bacterium isolated from nitrogen-deficient seedlings was found to be capable of producing some of the typical symptoms of sweet-corn wilt.

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CANKER DEVELOPMENT BY CRONARTIUM RIBICOLA ON YOUNG PINUS STROBUS

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INTRODUCTION

The blister-rust fungus, *Cronartium ribicola* Fischer, is found throughout most of the natural range of northern white pine, *Pinus strobus* L. This species has been planted extensively during reforestation activities; consequently, attention has been directed toward the development and effects of blister rust on young trees. Information concerned with the development of blister-rust cankers on northern white pine of transplant and young plantation age is considered valuable to those workers concerned with determining the age of infections, the time of fruiting, and the probable condition of infected trees at definite intervals following the establishment of the rust. Such information is available for older trees of *P. strobus* (6) and *P. monticola* Dougl. (5). The purpose of this paper is to contribute additional information of this nature on young trees of *P. strobus*.

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[1] These investigations were made by the writer while employed as agent in the Division of Plant Disease Control, Bureau of Entomology and Plant Quarantine, and the Division of Forest Pathology, Bureau of Plant Industry, in cooperation with the New York State College of Forestry, Syracuse, New York.

METHODS

At Warrensburg, New York, from 1928 to 1933, inclusive, and at Syracuse, New York, in 1935 and 1936, a minimum of 1500 3- to 4-year-old pines were planted in 6-inch paper pots in April of each year. These were set in the ground in storage beds where they were protected from blister-rust infection according to standard control practices recommended by the Division of Plant Disease Control. In the early summer, when telia of *Cronartium ribicola* had developed on the leaves of certain bushes of cultivated European black currant, *Ribes nigrum* L., 20 to 30 potted pines per day throughout each summer were placed close beside the infected bushes for 12 or 24 hours. Thus every tree was subjected to possible inoculation by the rust for 1 period of at least 12 hours.

After exposure to the rust, the pines were planted permanently in plantations surrounded by a protection strip 900 feet in width from which ribes had been eradicated, a measure that resulted in less than 0.5 per cent introduced infection. The pines were planted without being removed from the pots, inasmuch as the paper pots soon became soft from the soil moisture and readily decayed. Thus the root systems were not disturbed or hampered in their development. In the plantations the scheme for planting was such that the position of each pine in a row was a key to the time and recorded conditions of exposure to the rust-infected ribes. Therefore, the exact age of each resulting infection as well as the prevailing weather conditions at the time of infection could be readily determined.

While in the storage beds, the potted pines were subject to the same soil and meteorological conditions as transplants might have been in a plantation. The pots were sufficiently large to accommodate the root systems and strong enough to keep the soil and roots undisturbed during the process of exposing the trees to the rust fungus. Hence, the only artificiality that entered into the work was the transporting of the potted pines to the infected ribes bushes for natural inoculation by the rust and back again to the permanent planting site. Therefore, it may be assumed justly that the development of the rust on these pines was the same as it would have been on unpotted pines, subject to the same soil and meteorological conditions. This assumption was strengthened when it was found that, within nearby forests, canker development occurred similarly upon infected transplants and natural reproduction.

Beginning with the autumn of the season after the year of inoculation, each tree was examined for the first appearance of blister-rust cankers during September, October, and April to August, inclusive, for 3 or 4 seasons. Records were made of the condition of the cankers and the trees at each examination. More than 16,000 trees were used and approximately 1,000 cankers were found and studied. Only those cankers whose development was not interfered with by rodent and secondary fungus pests and for which there are complete data are considered in this study. Consequently, only relatively few of them proved useful for obtaining the data presented in this paper.

RESULTS

Relation of Canker Development to Position of Needle Spots

Cronartium ribicola becomes established on white pine through the needles (2, 4). At the point of initial infection a discolored spot, known as a needle spot, appears. Although needle spots caused by the blister-rust fungus are usually characteristic, they, nevertheless, can be confused with spots resulting from other causes. In heavy infections several needles in a single fascicle may bear needle spots, and it is then impossible to tell from which spot or spots a bark canker may have arisen. Thus, in this phase of the investigation, are included only those needle spots that occurred singly in the needle fascicle above a young canker.

It was possible to associate 49 needle spots with cankers that appeared in the bark the first autumn following the season of inoculation (Fig. 1), *i.e.*, 12 to 15 months after the needles became infected. The interval between 12 and 15 months represents the difference in time between early summer and autumn of the season of inoculation and not the interval during which there was visible evidence of the fungus in the bark. With few exceptions, discoloration of the bark was not seen until the last week of August or the first week of September the year following that of inoculation, regardless of whether the needles were infected in July, August, or September.

The greatest number of the 49 cankers that appeared in the bark within 15 months resulted from single needle spots located 2 centimeters or less from the bark. This suggests that the distance of a needle spot from the bark influenced to some extent the time, following needle inoculation, when the fungus showed the first macroscopic evidence of its presence in the bark. This suggestion is strengthened by the fact that the average size of the cankers was greater when the needle spots were 2 cm. or less distant from the bark tissue than when located at a more distant point. However, the distance of a needle spot from the bark is probably not the only factor governing the size of a canker at a definite time after needle inoculation, because it was discovered that some young cankers became visible in the bark at the base of the needle fascicles after the needles had fallen. Thus the fungus was in the bark tissues for some time prior to visible discoloration. It is not

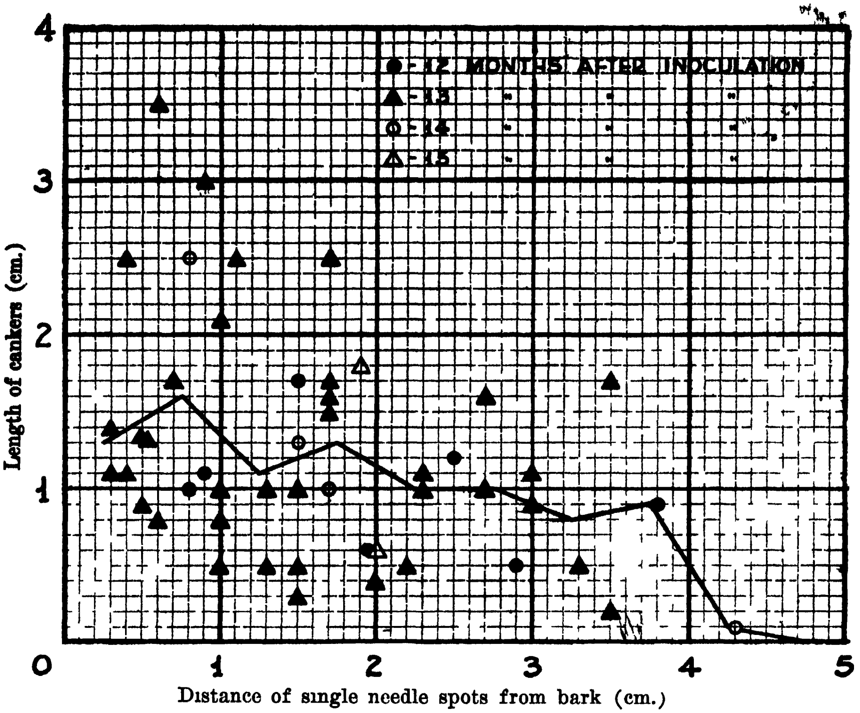

FIG. 1. Relation of the distance from the bark of single needle spots to the length of the resulting cankers 12 to 15 months after inoculation. The curve is drawn through the averages in each half-centimeter class, *i.e.*, 0.0–0.5 cm., 0.6–1.0 cm., 1.1–1.5 cm., etc.

known how soon discoloration occurs after the bark becomes invaded by the fungus nor whether the length of time that the fungus is in the bark is correlated directly with discoloration, which may account partly for the variation in the length of cankers of the same relative age as shown in figure 1.

Interval between Inoculation and Canker Appearance

As previously stated, the pines were examined first for infection in September and October of the season following the year of their exposure to the rust fungus and subsequently each spring and autumn for 3 or more years. A total of 547 cankers are considered in table 1. Of these, 274 or 50 per cent were visible the first autumn after the season of inoculation, that is, 12 to 15 months after needle infection; 239 or 44 per cent became visible the following spring; hence a total of 94 per cent of all of the cankers were visible the spring of the second season, approximately 20 to 23 months after the pines were exposed to the rust.

Three hundred eighty-seven cankers resulted from infection of year-old needles and 160 from current-season needles.[2] Forty per cent of the in-

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[2] It has been suggested (7) that the relative susceptibility of current season needles and year-old needles on potted pines may not be the same when they are inoculated with *Cronartium ribicola* the season they are potted as it would be on natural reproduction and on planted pines thoroughly established in the soil prior to inoculation. Studies to determine this point are now under way. Preliminary results indicate that the year-old needles are probably the more susceptible on the young potted pines, natural reproduction, and pines planted approximately 4 years before exposure to the rust.

TABLE 1.—*Number of blister rust cankers that appeared upon young white pines according to seasons following inoculation*

Year of inoculation	The appearance of cankers by seasons and according to age of infected needles										Total
	First year[a]		Second year				Third year				
	Fall		Spring		Fall		Spring		Fall		
	Current-season needles	Year-old needles	Current-season needles	Year-old needles	Current-season needles	Year-old needles	Current-season needles	Year-old needles	Current-season needles	Year-old needles	
1928	0	0	0	1	0	1	0	0	0	0	2
1929	1	4	0	0	1	1	0	0	0	0	7
1930	10	28	17	3	5	2	0	0	0	0	65
1931	27	53	5	38	0	0	0	1	0	0	124
1932	1	13	5	10	0	4	0	0	0	0	33
1933	9	32	12	53	8	2	1	0	0	1	118
1935	3	24	10	12	3	2	2	0	0	0	56
1936	11	58	29	44	0	0	0	0	0	0	142
Total	62	212	78	161	17	12	3	1	0	1	547

[a] The first year following that of inoculation.

fections in the current-season needles reached the bark the first season after infection and 59 per cent the second season. From the year-old needles 55 per cent became visible the first season and 44 per cent the second season. It has been the writer's experience that the larger percentage of the cankers from infected current-season needles quite commonly appear in the bark the second season after inoculation in contrast to the cankers resulting from infection of the year-old needles, the larger percentage of which can ordinarily be found the first autumn following the season of inoculation.

Although no cankers have been recorded here as visible prior to the first autumn following the season of inoculation, occasional cankers do appear by early summer. In 1938 a few cankers were found as early as June 22 on trees known to have been inoculated with the rust fungus between July and September of 1937. On July 13, 1938, a canker was found that resulted from needle inoculation on July 11, 1937. The needle spot was located 2 cm. from the bark and the canker was 0.6 cm. long.

Annual Extension of Cankers

When the rust becomes established in the bark of the branches it continues to extend toward the stem only so long as the bark between the cankers and the stem remains alive. In order to follow the development of the rust in the bark, only those trees upon which a single canker could be studied are considered here. Because all of the trees were about the same age (2–1 or 2–2 stock) they were of approximately the same size; hence, there was not enough variation in branch diameters to attempt to correlate canker enlargement with that factor. The diameters of the branches ranged from 0.2 to 0.5 cm. The extension downward from the point of infection of the bark was taken as the distance from the base of the infected needles to the yellow margin nearest the stem. The total growth for a season was considered to have been nearly completed by the end of September. The total growth downward at the end of the first year was taken as the distance from the base of an infected needle to the yellow margin by September 5 to September 15 of the autumn following the season of infection, *e.g.*, the total extension of the rust at the end of the first year on a tree inoculated in 1930 was taken as the distance to the yellow margin of the canker by September 1931; the second year, the growth downward by September 1932, etc.

At the end of the first year the total downward growth for 30 cankers averaged 0.7 cm.; the second year, 2.7 cm. for the same 30 cankers; the third year, 7.8 cm. for 20 of the cankers; and the fourth year, 7.3 cm. for 5 of the cankers. Since, for various reasons, all of the cankers measured the first year did not persist throughout the 4 years, continuous measurements were not possible on all of them. The average rate of growth downward for 30 cankers the first year was 0.7 cm.; for the same 30 cankers the second year, 2.0 cm.; for 20 of these cankers the third year, 5.0 cm.; and for 5 of the cankers the fourth year, 2.0 cm. The maximum rate of growth downward was approximately the same as that recorded for *Pinus monticola* Dougl. in wood of similar diameters, 0.1 to 0.2 in. (5, Table 9; 1). In 36 trees the rust reached the stem within 2 to 5 years, with an average of 3 years. It girdled the stems of 21 trees within 3.5 to 7 years, with an average of 4.5 years after the trees first became infected. The rust killed 19 trees within 4.5 to 9.5 years, or an average of 6.5 years after infection.[3] Trees with stem cankers that resulted from the fungus growing directly from infected needles to the stems naturally died in less time than those with branch cankers that eventually reached the stems. Likewise, trees with several branch cankers usually died sooner than trees with a single branch canker because of the multiple effect of the several branch cankers extending to and girdling the stems. By the time a branch canker in these young trees extended to the stem it necessarily produced a stem canker low in the crown or below it. Consequently, branch cankers that reached the stems almost always proved fatal to the trees, whereas direct stem infections were sometimes near the top of a long internode situated within the crown, so that when the stem was killed above the canker the internode died back to the node below before the rust could extend that far. As a result the rust could not survive and one of the primary branches then became a new leader, producing a tree free of blister rust (Fig. 2).

Production of Aeciospores

It has been indicated previously (3, 8) that on young infected trees of transplant size aecia may be produced within 3 years after infection, but the greatest number of cankers may not produce aecia until 4 years or more after infection has taken place. The results of the present study substantiate this conclusion. Thirty infected trees that remained alive for 7 or more years after infection produced aecia as follows: 2 years after infection, 3 trees; 3 years, 4 trees; 4 years, 2 trees; 5 years, 15 trees; 6 years, 13 trees; and 7 years, 13 trees. Thus, according to the preceding data, aecia were produced mostly after the rust had become established in the stems.

Pycnial drops were commonly produced on cankers from June to September, beginning for the most part the second summer after the pines became infected. The presence of pycniospores did not necessarily insure aeciospore production. Aeciospores failed to develop for several reasons, such, for instance, as the following: that portion of the bark in which pycniospores had formed was often killed before the next spring by the girdling effects of the rust; rodents occasionally gnawed off that part of the bark where pycniospores had occurred; grasshoppers very commonly chewed away the pycnial blisters and exposed the underlying tissue to desiccation; and secondary infection of blister rust cankers by fungi, especially one of the Nectriaceae,[4]

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[3] It was impossible to make comparisons between equal numbers of cankers and infected trees throughout this phase of the work, since factors impossible to control so affected some cankers and trees as to necessitate dropping them from further consideration, *e.g.*, some cankers became parasitized; others died for unexplainable reasons; etc.

[4] Dr. John Ehrlich, Pathologist, School of Forestry, University of Idaho, has identified specimens as *Ophionectria cylindrospora* (Sollman) Berlese and Voglino: Syn. *Scoleconectria scolecospora* (Brefeld and Tavel) Seaver.

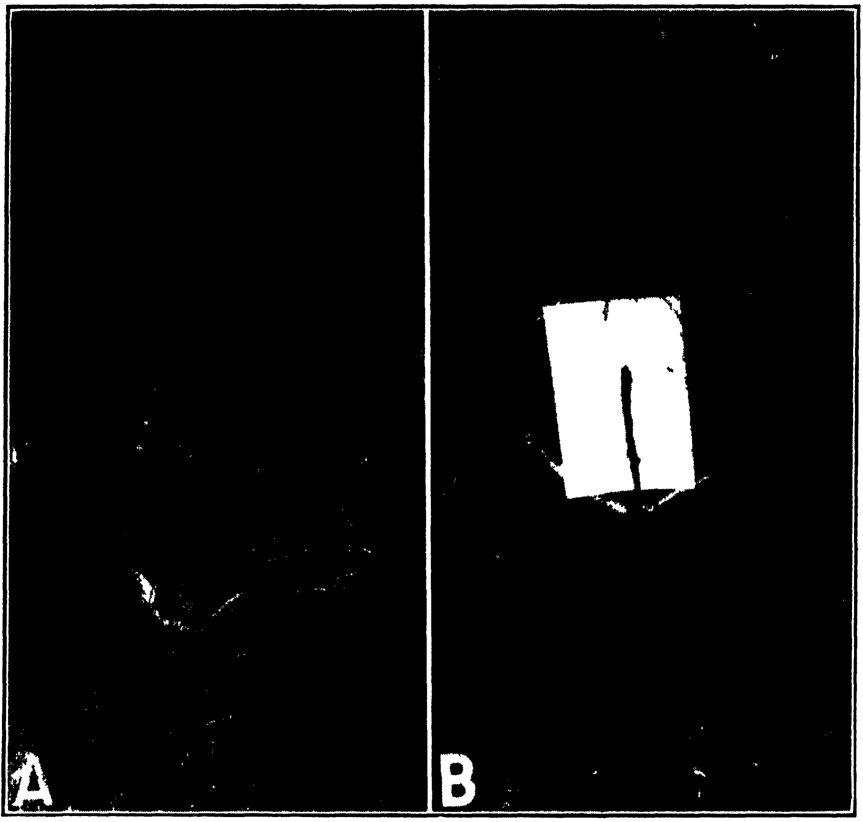

FIG. 2. Stem-cankered seedlings of *Pinus strobus* freed of the rust by the death of the infected leaders. A. A blister rust canker established in the stem in 1931 at the top of the 1930 internode. B. A canker established in 1933 in the 1933 internode. (In order to photograph the trees in 1938 it was necessary to remove some of the branches and twist others out of their normal position.)

often resulted in the death of the cankered areas and, consequently, the rust was killed.

CONCLUSIONS AND SUMMARY

Studies concerned with the development of blister rust cankers on young northern white pines were conducted under natural conditions within the Adirondack region and in central New York State.

It seems probable that to some extent the time necessary for the fungus to become evident in the bark depends on the distance of the needle spot from the bark tissue. This is indicated by the smaller size of the cankers resulting from single needle spots located at the greater distances. It is significant that needle spots located as much as 4 to 4.5 cm. from the bark can give rise to bark cankers 0.1 cm. in diameter within 12 to 15 months after initial infection, thus allowing the fungus from these spots to reach the bark even in dry seasons when these young trees may retain their needles for only 2 summers. Needles of *Pinus strobus* are normally retained by transplants and young plantation stock for 26 to 28 months, and a few needles for 36 to 38 months; thus time is provided for the fungus to reach the bark from some of the needle spots located at slightly greater distances than 4.5 centimeters in both current-season and year-old needles.

Approximately one-half of all the cankers which developed were visible by the fall of the season following that of inoculation; by the second spring after inoculation 94 per cent were visible. Therefore, by use of sample plots in any desired area it should be possible by September of each year to secure an idea of the intensity of infection for the preceding year, and by the following spring almost exact information.

The first appearance in the bark of the larger percentage of the cankers from current-season needles was somewhat more tardy than from the year-old needles, inasmuch as the majority of them appeared the second spring instead of the first autumn following the year of inoculation. This delay cannot be attributed logically to the distance of the needle spots from the bark because the average length of the current-season needles was appreciably less than that of the year-old needles during the greater part of each summer, and, consequently, it is probable that the average distance of the needle spots from the bark was less. The explanation may possibly be concerned with the maturity of the needles and the slight differences in tissue structure of the bark at the end of 1 and 2 years.

It was discovered that, in the cankers studied, the rate of extension of the rust down the branches toward the stem increased with the age of the cankers through the third year, ranging from 0.7 to 5.0 cm. On 36 trees the average time for branch cankers to extend to the stem was 3 years. Thus in young plantations which may become invaded by the rust but, subsequently, protected by accepted control practices, it is possible to detect branch cankers sufficiently soon so as to remove the infected branches on many of the trees before the stem is invaded and thereby free the infected trees of the disease when it is feasible to do so. After the stems become infected from the branch cankers the trees usually succumbed to the disease within an average of 3.5 years although some trees persisted for 6.5 years.

The production of aeciospores occurred in a few branch cankers, but aeciospore production was commonly delayed until the cankers had become established in the stems for a year or more. However, due to numerous factors, aeciospore production occurred on relatively few of the originally infected trees.

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PHYTOPATHOLOGICAL NOTE

Scirrhia acicola (Dearn.), n. comb., the Perfect Stage of the Fungus Causing the Brown-spot Needle Blight of Pines.[1]—One of the obstacles to the natural and artifical reproduction of longleaf pine, *Pinus palustris* Mill., is the brown-spot needle disease. Athough the fungus causing this disease usually has been referred to as *Septoria acicola* (Thüm.) Sacc., the writer believes that *Lecanosticta acicola* (Thüm.) Syd. is the more tenable binomial for its imperfect stage.[2]

The connection previously unproved between the imperfect stage and the ascigerous fungus, *Oligostroma acicola*[3] Dearn., was determined by studying 18 single-ascospore cultures obtained from 4 collections of needles from 2 host species. The development of the monoascosporic cultures has been watched for 6 to 14 months, and all are macroscopically similar to colonies started from single conidia of *Lecanosticta acicola.* The conidia formed in each ascosporous culture are similar in shape, size, and color to the spores formed in conidial cultures. Cultural studies show that the physiological response of the ascospores to external factors affecting germination and early hyphal growth is approximately the same as that of conidia taken directly from pine hosts. In addition, ascogenous locules often develop marginally in the old stromata of the conidial stage of the brown-spot fungus on needles.

Study of the compact, non-clypeate, erumpent stroma of the ascigerous fungus shows that its disposition in the genus *Oligostroma* Sydow is untenable. In *Oligostroma proteae* Syd. on *Protea flanigani*, 1915 (A. Pegler, 1899), the generic type for *Oligostroma,* non-erumpent ascocarps formed in the leaf tissue are connected at their apices with a clypeus. Because of similarity in stromatic structure the ascigerous fungus is here referred to the genus *Scirrhia* Fckl. and an emended description is appended as follows:

Scirrhia acicola (Dearn.), n. comb. Syn. *Oligostroma acicola* Dearn. Mycologia 18: 251, 1926.

Stromata, linear, never effused, innate-erumpent, sometimes greatly reduced, covered by the epidermis, usually seated in the mesophyll, maximum size 2.5 × .3 mm. Locules 1–18 in a stroma, mostly in a single row, sometimes in 2, rarely in three rows, usually conical globose, but variable in shape, depending on the tissue of the host, 40–80 μ in diameter. Not distinctly ostiolate although there are sometimes indications of ostioles. Asci aparaphysate, 30–35 × 6–9 μ, tapering apically, 8-spored. Spores hyaline, obliquely uniseriate or biseriate near the base of the asci, one-septate past the center, non-constricted, oblong-cuneate, typically with oil globules, 9–16 × 3–4 μ.

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[1] In the taxonomic phase of this investigation the writer acknowledges assistance from Wm. W. Diehl and J. A. Stevenson of the Division of Mycology and Disease Survey, Bureau of Plant Industry, Washington, D. C.

[2] Details of the taxonomy and synonomy of the imperfect stage will be published later.

[3] Dearness, John. New and noteworthy fungi—V. Mycologia 20: 235–246. 1928.

On Abietineae: *Pinus palustris* Mill., Fla., Ga., La., N. C.; *Pinus taeda* L., Ark., Texas; *Pinus thunbergii* Parl., Fla.

Type locality: Silver Springs, Fla., on *Pinus palustris.*

Distribution: North Carolina to Texas and inland to southern Arkansas.

Specimens examined: Herbarium, Div. of Forest Pathology, Bureau of Plant Industry, Washington, D. C., on *Pinus palustris,* Helena, Ga., coll. G. G. Hedgcock, F. P. 17627; Durham, N. C., coll. Carl Hartley, F.P. 50000; Woodworth, La., coll. Paul V. Siggers, F.P. 50001; Urania, La., coll. Paul V. Siggers, F.P. 50002; Bogalusa, La., coll. Paul V. Siggers, F.P. 50003; on *Pinus taeda,* Fordyce, Ark., coll. Dale Chapman, F.P. 50004; Rusk, Texas, coll. P. A. Young, F.P. 50005; on *Pinus thunbergii,* Camp Pinchot, Fla., coll. Paul V. Siggers, F.P. 50006.

The structure of the ascocarp suggests that the ascospores are ejected forcibly and disseminated by wind. There is also indirect evidence from a study of the dissemination of the fungus in nature, indicating that the perfect stage of the brown-spot fungus provides a method of aerial dissemination for a fungus previously known to have conidia adapted to dissemination by rain splash only. It is believed that the ascospores are responsible for the infections found far above the ground on saplings and mature trees. There is evidence also that the perfect stage of the brown-spot fungus appears only after death of most of the needle and that the ascospores are produced mainly in winter and spring.—PAUL V. SIGGERS, Division of Forest Pathology, Bureau of Plant Industry, in cooperation with the Southern Forest Experiment Station, New Orleans, La.

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Joint authorship indicated by pages in ( )

Prepared by Frederick V. Rand, Office of Experiment Stations, U. S. Department of Agriculture

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Dr. L. R. Jones has very kindly presented to the American Phytopathological Society, through the Committee on Donations and Legacies, 500 copies of his memoir entitled "Biographical Memoir of Erwin F. Smith."

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